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STRIPE RUST (PUCCINIA GLUMARUM) OF CEREALS AND GRASSES IN THE UNITED STATES [1]

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INTRODUCTION

Stripe rust was first reported from Europe by Schmidt (*22*) [3] in 1827. He described the fungus as *Uredo glumarum*. In 1894, Eriksson and Henning (*4, p. 197*) reported their discovery of the telial stage of the fungus, and transferred the species to the genus Puccinia. This, summarized, becomes *Puccinia glumarum* (Schm.) Erikss. and Henn. Prior to 1894, the fungus had been confused with *Puccinia rubigo-vera* DC., *P. dispersa* Erikss. and Henn., *P. graminis* Pers., and *P. coronata* Corda, specimens of which were deposited in several herbaria under the name *P. glumarum*. In North America, specimens 1471a and 1471b of the Ellis and Everhart Collection of North American Fungi, and referred to by them as *P. glumarum*, have since been found to have been erroneously classified. Likewise this is true of Ravenel's specimen No. 100 of his *Fungi Caroliniani exsiccati*. Rabenhorst's specimen No. 3214, collected in North America, the writers have not examined, but Sydow and Sydow (*23*) have questioned the correctness of its classification as *P. glumarum*. An examination of specimens in the herbarium of the New York Botanical Garden established the fact that *P. glumarum* was collected near Seattle and Everett, Wash., as long ago as June, 1892, when C. V. Piper reported it as *P. rubigo-vera* on *Elymus glaucus* Buckl. (*E. americanus* Vasey and Scribn.) and a month later on *Bromus carinatus hookerianus* (Thurb.) Shear.

Other American collections, made prior to 1915, are listed farther on under *Exsiccati*. It should be noted here, however, that an examination of rust specimens contained in the Arthur Herbarium, La Fayette, Ind., brought out the fact that in August, 1911, E. and E. T. Bartholomew collected *P. glumarum* on *Sitanion hystrix* (Nutt.) J. G. Smith. This collection was distributed as *Puccinia agropyri* No. 4611. In the specimen envelope, attached to the herbarium sheet, was found the following interesting note to Dr. Arthur in E. Bartholomew's handwriting:

> This differs so radically from what you have been calling *P. rubigo-vera* on this host as found in Iowa, Nebraska, and Kansas, that I am sending it to you for examination. Collected in an old, previously cultivated field where there is no hint of aecial infection. I have called it provisionally *P. glumarum* (Schm.) Erikss. and Henn., on *H. jubatum*, Rock River, Wyoming, August 24, 1911.

Thus it is seen how close Bartholomew came to definitely recognizing stripe rust in America in 1911.

Although stripe rust in the United States was not certainly identified as such until May, 1915, when it was

[1] Received for publication July 1, 1924—issued January, 1925. It was originally intended to make of this paper a comparative study on the occurrence of *Puccinia glumarum* in the United States and Europe. That part of the proposed paper relating to this rust in Europe was to have been contributed by the late Dr. F. Kølpin Ravn, of Denmark, as joint author. Owing to Dr. Ravn's untimely death this plan was abandoned. However, the notes made in the United States in connection with Dr. Ravn's trip here during 1915 are included because of their historic interest.

[2] The authors wish to make grateful acknowledgment to Dr. A. S. Hitchcock and Mrs. Agnes Chase for assistance in identifying the various grasses named in this paper, and to Dr. Ruth F. Allen, Mrs. Rose E. Gamble, and J. M. Shull for the preparation of certain of the illustrations. The authors are indebted to the Office of Pathological Collections, Bureau of Plant Industry, United States Department of Agriculture, the New York Botanical Garden, Harvard University, the University of Minnesota, and the Washington and Purdue University Agricultural Experiment Stations for placing at their disposal the facilities of their several herbaria.

[3] Reference is made by number (italic) to "Literature cited," p 226–227.

specifically recognized by F. Kølpin Ravn, there exists in certain herbaria of the country convincing evidence of the existence of this rust in the United States for a period of at least 23 years prior to its discovery. Indeed, it is possible that stripe rust established itself in western North America centuries ago. Its occurrence on certain grasses indigenous to Alaska has led the writers to consider more fully the distribution of the rust by these hosts up and down the Pacific Coast and throughout the tributary intermountain territory.

During the summer of 1916 the writers made numerous collections of stripe rust on wheat and certain wild hosts in Skagit and San Juan Counties, Wash. Here they observed for the first time the occurrence of this rust on *Bromus pacificus* Shear and *B. sitchensis* Bong. Its occurrence on *B. marginatus* Nees had first been noted in June, 1915, at Pullman, Wash. Infected plants of *Hordeum jubatum*, *H. nodosum* L., and *Elymus glaucus* Buckl. have been collected frequently, the first collection on these hosts, as elsewhere noted, being that of E. and E. T. Bartholomew in 1911. *H. caespitosum* Scribn., *Agropyron spicatum* (Pursh) Rydb. and *A. violaceum* (Hornem.) Lange also are congenial hosts, and infected specimens have been collected by C. W. Hungerford.

All of the hosts of *P. glumarum* recorded in the foregoing paragraph are, according to Hitchcock, common to Alaska. Although the writers have no record of the occurrence of this rust on any of these grasses in Alaska, it seems likely that it may be found there. The fact that it has been observed on certain of these hosts in such isolated localities as San Juan and Vancouver Islands would tend to support the assumption that this rust occurs also in Alaska. And if it be present in Alaska, it seems not unreasonable to infer that it may have reached this continent from Siberia by way of Kamchatka and the Aleutian Islands, or during an earlier period, while the two continents were yet one.

COMMON NAME

The disease caused by *Puccinia glumarum* is commonly known among most British botanists and plant pathologists as "golden rust," though occasionally it is referred to as "yellow rust." In Germany and Austria it is known as *Gelbrost* (yellow rust), while in Sweden and Denmark it is called *Gulrost*. According to Ferraris (*5*), stripe rust is commonly known in France as *rouille jaune*, and in Italy as *ruggine striata del grano*. Thus it is noted that in all cases, excepting that of Italy, the common name is based solely on a color character.

To the writers it has seemed that the more descriptive common name, stripe rust, should be adopted, for certain other of the cereal rusts might quite properly be styled yellow or golden and thus prove confusing to the inexperienced student or observer. As an example of the need of greater attention to the descriptiveness of common names, the writers might cite their experience in attempting to determine just what rust is referred to by hundreds of voluntary crop reporters who record outbreaks of "rust," "red rust," "black rust," "yellow rust," etc. Many of the reports merely state "rust" and for this reason are of very doubtful, if, indeed, of any, value to the student interested in a specific rust.

For this disease, therefore, the writers propose the general adoption of the name stripe rust. This is believed to be preferable to the name yellow rust, not only for the reason that it is more definitely descriptive but also that it has the merit of at once enabling the observer to distinguish it from all other cereal rusts.

THE DISCOVERY OF STRIPE RUST IN THE UNITED STATES

Stripe rust was discovered in the United States and definitely determined as such by the late Dr. F. Kølpin Ravn, of Copenhagen. On May 25, 1915, while examining wheat fields near Sacaton, Ariz., he observed and collected the rust, recognizing it on the spot as *P. glumarum*. At the time of the discovery of this rust, the wheat near Sacaton was in the dough stage of development and no teliospores were observed. This would seem to indicate a possible late or delayed initial infection, for it should be added that in none of the plants observed was there evidence of more than a mild attack.

On the same day, May 25, 1915, one of the authors (Johnson) collected what was later identified as *Puccinia glumarum* on *Hordeum murinum* L. near Tehachapi, Kern County, Calif. Although a diligent search was made for the rust in the wheat fields of the lower Sacramento Valley and elsewhere in California during that year, it was not found. On June 10, of the same year, it was observed again at Corvallis, Oreg., by Dr. Ravn and others who noted its occurrence on Baart and

Chul wheat. Later in the same month it was found in abundance at Moro, Oreg., a district of relatively scant rainfall, and at other points in Washington and Idaho. These observations are referred to later.

DISTRIBUTION

Stripe rust is widely distributed throughout Europe, though its ravages are confined chiefly to the northern countries, that is, Great Britain, Sweden, Norway, Denmark, Belgium, France, Russia, and Austria. It is prevalent also in Egypt, Algeria, Japan, and India, but has not yet been reported from South America or from New Zealand or Australia.

Although authenticated reports of its occurrence in eastern Siberia are not at hand, its presence in western Siberia (Akmolinsk) and in Japan supports the belief that this rust may have become established on the mainland adjacent to Japan.

In North America, stripe rust thus far has been observed eastward to the Black Hills of South Dakota, northward as far as Duncan, British Columbia, and southward as far as Mexico City, Mex. Its distribution on this continent has not extended eastward beyond 103° W. longitude, notwithstanding the fact that in addition to the cultivated hosts, wheat, barley, and rye, it occurs naturally on at least 34 wild grasses, 13 of which extend over a wide range of territory east of the one hundred and third meridian. *Hordeum jubatum*, one of its most congenial hosts, now extends across the continent, yet the writers find it is not infected with *Puccinia glumarum* east of what may be designated the Rocky Mountain formation. Other wild-grass hosts, the distribution of which extends eastward beyond the Pacific and intermountain States, are *Agropyron spicatum*, *A. cristatum* (L.) Gaertn., *A. dasystachum* (Hook.) Scribn., *A. desertorum* (Link) Schult., *A. intermedium* (Host.) Beauv., *A. violaceum*, *Bromus rubens* L., *Elymus canadensis* L., *E. glaucus*, *E. striatus* Willd., *E. virginicus* L., *Hordeum nodosum*, *H. pusillum* Nutt., *Hystrix patula* Moench and *Sitanion hystrix*.

Just what factor or combination of factors has operated to prevent the march of stripe rust, if not simultaneously with, at least in the wake of, such hosts as have established themselves within the Mississippi Valley and beyond, has not yet been determined. It may be that it is slowly advancing eastward and that it is merely a matter of time before a visitation of this pest will befall the wheat fields of the Great Plains and the fertile prairies of Iowa, Minnesota, and Illinois. And yet, the writers' records show that *P. glumarum* certainly has been present in Wyoming since 1911. During the decade that has elapsed, there have been propitious rust years, but these have not extended the disease beyond the Black Hills in western South Dakota where, in August, 1919, it was observed by one of the authors (Johnson) on cultivated barley. In spite of repeated attempts to find it in this locality in subsequent seasons, it has not been observed on either cultivated or wild hosts.

The fact that the map (fig. 1) shows no recorded observation of this rust in either Nevada or New Mexico is not to be interpreted as evidence of the absence of *Puccinia glumarum* from those States. It is not unlikely that a more thorough and extensive survey, such, for example, as has been conducted in Oregon and Washington, would have resulted in its discovery in both Nevada and New Mexico.

EXSICCATI

Fungi Bohemici. 155, *Triticum repes*, Bohemia, May, 1898.

Eriksson, *Fungi Parasitici Scandinavici*. 425 (II, *Hordeum vulgare*, Sweden, July, 1894); 426 (*Hordeum jubatum*, Sweden, Aug., 1892); 427 (*Hordeum maritimum*, Sweden, Sept., 1894); 428a and 428b (II, III, *Triticum vulgare*, Sweden, June, 1890); 429 (II and III, *Triticum vulgare*, Sweden, Oct., 1894); 430 (II, *Elymus arenarius*, Sweden, Aug., 1894).

Sydow *Uredineen*. 1070 (II, *Secale cereale*, Germany, Feb., 1896); 1591 (*Hordeum hexastichum*, Germany, June, 1901); 2519 (II and III, *Triticum caninum*, Germany, July, 1912); 2467 (II, *Triticum repentis* L., Denmark, June, 1912); 682 (*Triticum repens*, Germany, July, 1892); 883 (II, *Hordeum vulgare*, Germany, 1894. Issued as *Puccinia rubigo-vera*).

Krieger, *Fungi Saxonici*. 1406 (II, *Triticum vulgare* bei Koenigstein, 7–1895); 1407 (II and III, *Triticum vulgare*, Germany); 1408 (II, *Secale cereale*, Germany, 7–1895); 1452 (II, *Triticum caninum*, Schweiz, Juni, 1899); 2303 (III, *Triticum caninum*, Koenigstein im Bielatale, 8–1914).

Krieger, *Schaedliche Pilze unserer Kulturgewaechse*. 62 (II and III, *Triticum vulgare*, Germany, July, 1895); 63 (II, *Triticum vulgare*, Germany, July, 1895).

Petrak, *Flora Bohemiae et Moraviae exsiccata*. 376./a (II, *Secale cereale*,

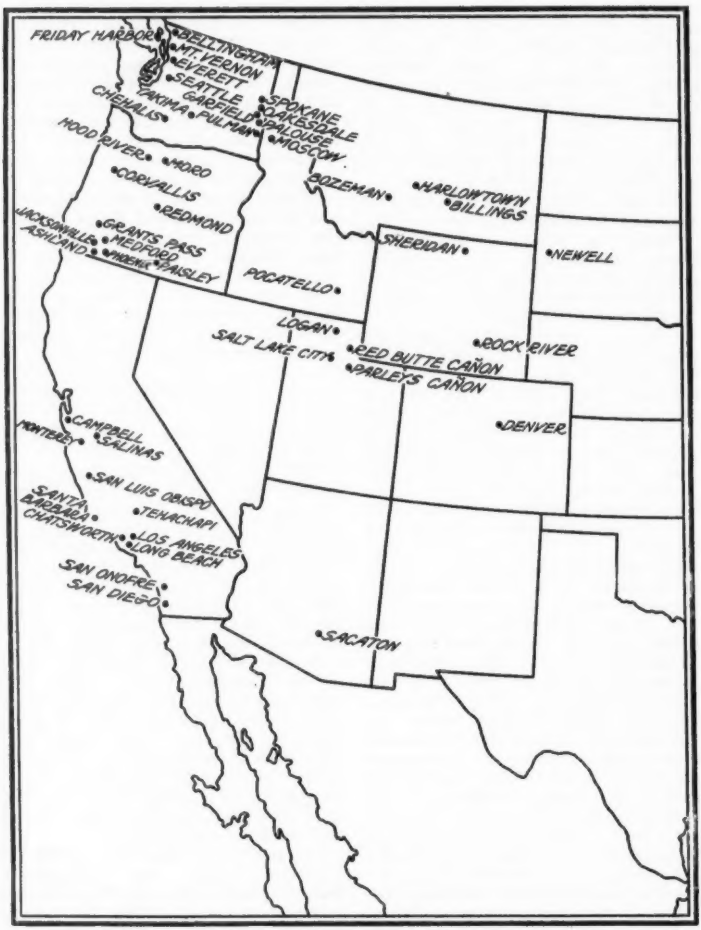


FIG. 1.—Map showing present known distribution of stripe rust in the United States

Boehmen, 6, 1894); 376./b (II, *Calamagrostis epigeios*, Moravia, 9–1912).

Fungi Eichleriani. 104 (*Hordeum vulgare*, Moravia, May, 1906); 105 (*Triticum aestivum*, Moravia, June, 1906).

Saccardo, *Mycotheca italica*. 1445 (II, III, *Secale cereale*, Italy, May, 1904).

Bucholtz and Bondarzew, *Fungi Rossiei exsiccati*, Ser. A, 163 (*Hordeum vulgare*, Russia, July, 1917).

Butler, E. J. (*Hordeum jubatum*, India, Feb., 1905).

Herb. Crypt. Ind. Orient. (II, *Hordeum vulgare*, India, April, 1904); (II, *Hordeum vulgare*, India, 1907); (II, III, *Hordeum vulgare*, India, March, 1903).

Westendorp et Wallays, *Herbier Cryptogamique*. 1845–1859. 231 ("leaves of cereals," Belgium. Issued as *Uredo rubigo-vera*).

Plantes cryptogames de France. 1476 (II, III, *Triticum* sp., France, 1845–1859. Issued as *Uredo glumarum*); 568 France, 1845–1859. Issued as *Uredo glumarum*).

Bartholomew, *North American Uredinales*. 1063 (III, *Hordeum jubatum*, Wyoming, Aug., 1911. Issued as *P. montanensis*); 2758 (III, *Agropyron smithii*, Colorado, Aug., 1916); 2435 (*Elymus glaucus*, California, June, 1919); 1755 (*Hordeum nodosum*, Colorado, Aug., 1916).

Bartholomew, *Fungi Columbiani*. 3763 (*Hordeum jubatum*, Wyoming, Aug., 1911. Issued as *P. montanensis*); 4369 (*Hordeum jubatum*, Montana, Aug., 1913. Issued as *P. rubigo-vera*); 4611 (*Sitanion elymoides*, Wyoming, Aug., 1911. Issued as *P. agropyri* (?)).

Garrett, *Fungi Utahenses*. 138 and 139 (II and III, *Elymus glaucus*, Utah, June, 1907. Issued as *P. rubigo-vera*); 191 (II and III, *Hordeum jubatum*, Utah, July, 1919. Issued as *P. rubigo-vera*); 192 (II and III *Hordeum pusillum* Utah, Aug., 1909. Issued as *P. rubigo-vera*).

Piper, *Washington Flora*. 41 (II, *Elymus Americanus*, Wash., June, 1892. Issued as *P. rubigo-vera*); 206 (*Bromus Hookerianus*, Wash., July, 1892. Issued as *P. rubigo-vera*).

Strand, *Flora of Montana*. 180 (II, *Hordeum jubatum*, Montana, Oct., 1914. Issued as *P. agropyri*).

Flora of Oregon. [Oregon Agricultural College.] 1385 (II, *Hordeum gussoneanum*, Oregon, June, 1914. Issued as *P. rubigo-vera*); 1423 and 1429 (*Sitanion hystrix*, Oregon, July, 1914. Issued as *P. agropyri*); 1596 (*Elymus glaucus*, Oregon, May, 1914. Issued as *P. agropyri*).

Ex Herb. Bethel. (*Hordeum jubatum*, Colorado, June, 1916. Issued as *P. agropyri*); (III, *Hordeum jubatum*, Colorado, July, 1916. Issued as *P. agropyri*).

Ex Herb. Holway. 3067 (*Hordeum jubatum*, Mexico, Oct., 1898. Issued as *P. rubigo-vera*).

ECONOMIC IMPORTANCE OF STRIPE RUST IN THE UNITED STATES

From present knowledge of the great economic importance of stripe rust in Europe, especially on wheat, and from the evidence of its virulence in the United States, particularly as noted on certain wheat varieties, it is not improbable that under optimum conditions its ravages might prove a serious menace to wheat culture on this continent.

It is more fully brought out later that the severity of the attacks on different organs of the wheat plant varies markedly with the variety. For example, in certain varieties of wheat, especially Chul, which is grown to a limited extent in the Western States, the attack is very severe on the glumes and kernels and rather indifferent on the leaves. In varieties where this type of infection occurs, the results are frequently serious. The rusted kernels become greatly shrunken, their quality generally lowered, and the yield consequently much reduced (Pl. 1, *c*).

Possibly the most far-reaching importance of the attack of the disease on the kernels is the connection that this type of infection may bear to carrying the fungus over from one crop to the next. The importance of the attack on the glumes and stalks is also referred to by Blaringhem (*3*) who records the greater injury from this type of attack.

In most varieties of wheat, stripe-rust infection is confined chiefly to the leaves. The abundance of such infection may vary greatly. In cases where it is severe, 85 to 100 per cent, there no doubt results noticeable and serious injury.

Because of its possible menace to wheat culture in the extensive and more humid wheatlands of the Mississippi Valley and of the Atlantic States, it is unquestionably important that more be learned about the life history of this rust; its present geographic distribution; its behavior and effects on different hosts and their activities; its seasonal

cycle; and the conditions which favor or inhibit its development. Above all, it is important that the utmost effort be made to confine this rust to its present known range in the Western States, for if one may judge of its possible behavior under conditions of greater humidity and more favorable temperature, from what is known of its ravages in Europe, its importance as a limiting factor in the production of wheat would scarcely be second to that of stem rust.

HOSTS

Not unlike *Puccinia graminis* Pers., *P. glumarum* is found on a relatively large number of both wild and cultivated Gramineae. Investigation has shown that definite specialized forms exist, such, for example, as those common to wheat and barley which, according to Eriksson and Henning (*4*), are sharply fixed. In Europe, where this rust has long been common, the following hosts have been recorded: *Calamagrostis epigeios* (L.) Roth, *Elymus arenarius* L., *Hordeum jubatum*, *H. vulgare* L., *Aegilops triuncialis* L. (*Triticum triunciale* (L.) Rasp.), *Secale cereale* L., *T. vulgare* Vill., *T. turgidum* L., *T. spelta* L., *T. repens* L. (*Agropyron repens* (L.) Beauv.), *T. polonicum* L., *T. giganteum* Roth, *T. durum* Desf., *T. monococcum* L., *T. dicoccum* Schrank, *T. desertorum* Fisch. (*Agropyron desertorum* Schult.), *T. compactum* Host., *T. caninum* Ledeb. (*Agropyron caninum* L.), and *Dactylis glomerata* L. Doubtless it has other hosts among the wild grasses of Europe, Asia, and Africa, such, for example, as other species of Agropyron, Bromus, Elymus, and Hordeum, but record of such is absent from the literature seen. One species particularly, *Agropyron cristatum* (L.) Gaertn., occurring naturally in Europe, and especially in Russia, has proved a most congenial host of *P. glumarum* in America, though it is not reported as such in Europe.

In North America, stripe rust thus far has been reported as occurring naturally on the following hosts: *Agropyron spicatum* (Pursh) Scribn. and Smith, *A. cristatum* (L.) Gaertn., *A. dasystachum* (Hook.) Scribn., *A. intermedium* (Host.) Beauv., *A. violaceum* (Hornem.) Lange, *A. lanceolatum* Scribn. and Smith (*A. riparium* J. G. Sm.?), *A. desertorum* Schult., *Bromus marginatus* Nees, *B. pacificus* Shear, *B. sitchensis* Trin., *B. carinatus* Hook. and Arn., *B. carinatus* v. *hookerianus* (Thurb.) Shear, *B. rubens* L., *B. brizaeformis* Fisch. and Mey., *B. polyanthus* Scribn., *Elymus canadensis* L., *E. condensatus* Presl., *E. glaucus* Buckl., *E. macounii* Vasey, *E. striatus* Willd., *E. virginicus* L., *Hordeum caespitosum* Scribn., *H. jubatum* L., *H. gussoneanum* Parl., *H. murinum* L., *H. nodosum* L., *H. pusillum* Nutt., *H. vulgare* L., *Hystrix patula* Moench, *H. californica* (Boland.) Kuntze, *Phalaris paradoxa* L., *Sitanion jubatum* J. G. Smith, *S. hystrix* (Nutt.) J. G. Smith, *S. longifolium* J. G. Smith, *Secale cereale* L., *Triticum vulgare* Vill., *T. polonicum* L., *T. compactum* Host., *T. durum* Desf., *T. spelta* L., and *T. dicoccum* Schrank.

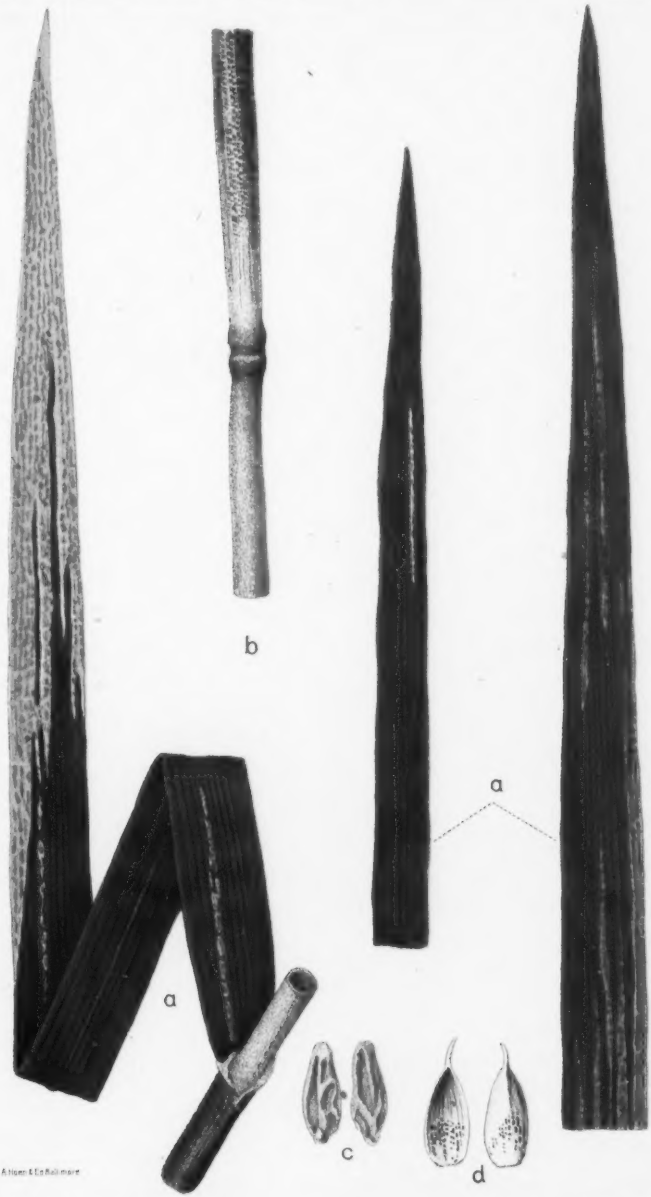
Thus far the writers have failed to note the occurrence of stripe rust on *Dactylis glomerata* in America, nor has it been possible for them to produce infection by artificial means, in spite of conditions highly favorable to inoculation and subsequent incubation. These facts would seem to indicate that there are inherent differences between the European and American forms of the rust or that the European and American forms of the host are not entirely identical.

DESCRIPTION OF STRIPE RUST

The aecial stage of *Puccinia glumarum* is unknown. Eriksson and Henning (*4*) report inoculation experiments with teliospores on various species of Boraginaceae, but in all instances they obtained only negative results. A further and more exhaustive study, however, may result in the discovery of the aecial host. Because one has not yet been found, is not sufficient justification for assuming its nonexistence, or that the rust, having formerly required such a host, may, under less exacting conditions, have lost its capacity for aecial development.

EXPLANATORY LEGEND FOR PLATE 1

a, Leaves with characteristic stripelike lesions; *b*, portion of culm showing fine stripes of telia; *c*, shriveled kernels from head of a severely rusted plant; *d*, ventral and dorsal views of glumes showing telia.


b
a
a
c
d

ON WHEAT

On the leaves of adult wheat plants, the uredinia develop in end-to-end series between the vascular bundles, forming long stripes, sometimes extending throughout the length of the leaf. Many of these, developing side by side, seem to coalesce, thus completely transforming one or the other, or both, surfaces of the leaf to a mass of uredinia. Most commonly the rust occurs on the leaf blades, but it appears not infrequently also on the leaf sheaths, and in certain peculiarly susceptible varieties of wheat the glumes, awns, and kernels are severely attacked. On the leaf blades of adult plants, the uredinia are irregularly scattered and strikingly characteristic as to form and color. In general appearance they are much the same on both sides of the leaf. They are narrowly oblong to linear in form, sharply delimited laterally and less so at the ends (Pl. 1, *a* and *b*). Individually, they vary in extent from about 1 to 3 mm. in width and from 10 to 110 mm. in length. When urediniospores alone are present, the color of the stripelike lesions is a reddish orange yellow when the uredinia are open and lemon yellow when they are not open. Mikado orange to capucine yellow, according to Ridgway (*21*).

On the more susceptible varieties, where uredinia are numerous on the same leaf blade, the attacked areas not uncommonly coalesce, either laterally, or end-to-end, or both, to such an extent that practically the entire leaf blade, or large portions of it, may be involved. On the sheaths, similar stripelike lesions occur, but they usually are fewer and less conspicuous. On the glumes, the attack is strikingly characteristic, especially on certain varieties of wheat which are apparently more susceptible than others in these organs.

When the wheat heads are normally green, those attacked by the rust take on varying shades of yellowish green because of deficient chlorophyll in affected glumes. The number and distribution of glumes attacked on individual wheat heads may vary considerably, from a single glume to several widely scattered. In those heads where infection is most general and pronounced, all the glumes may be rusted. The uredinia and telia develop almost entirely on the ventral side of the glume, thus causing the color and general appearance of rusted heads to vary according to the severity of the infection. In milder cases only slight discoloration is noticeable. Instances of severe infection are characterized by marked discoloration and an abundance of free urediniospores which accumulate in such numbers as to form a yellow powdery mass on the inner surface of the glumes and over the developing kernel (Pl. 1, *d*). Likewise, the kernels vary markedly in appearance, depending on the severity of attack. When there are but few uredinia on the glumes, there usually is little or no shrinkage of the kernels; but when the infection develops early and becomes severe, the damage is fully as marked as that resulting from a like infection of stem rust (Pl. 1, *c*). When such kernels are sectioned and examined with the microscope they usually are found infected to a greater or less extent. In such infected kernels, the spores are produced in characteristic pockets in the pericarp as shown in Figure 2, and also by Eriksson and Henning (*4*, *Pl. 9*).

In seedling infection, the uredinia appear in more or less rounded patches without any seeming tendency to form stripes. Indeed, in this stage of its development stripe rust does not differ markedly from orange leaf rust (*Puccinia triticina* Erikss.), except that in the latter the distribution of the uredinia is more general over the surface of the leaf, and the color of the spores in mass is darker yellow, more typically orange in color. This noticeable difference between the disposition and character of the uredinia on the leaves of adult and seedling plants is to be explained on purely anatomical grounds, as has been pointed out by Eriksson and Henning (*4*). Structurally, the seedling leaf is sparingly supplied with vascular tissue and is markedly succulent (fig. 3, *A*). The relative absence of vascular tissue contributes to the easy and general spread of the mycelium and the irregular eruption of the surface as outlets for the urediniospores. In the older or adult leaves, the vascular bundles check the spread of rust laterally and confine it to rather sharply defined areas, usually

many times longer than wide (fig. 3, B). Thus, if infection obtains near the tip or distal end of a leaf, the affected area widens as the fungus extends toward the base of the leaf.

The telial stage is not at all uncommon, especially later in the crop season. It may appear in any organ or structure of the host attacked by the uredinial stage of the rust, namely, leaf blades, sheaths, stalks, glumes, and kernels. In each of these the telia become readily evident from their dark brown to black color. They may occur in some part, usually the older portions, of the uredinial lesions; or they may develop quite independently. On the leaves and stalks, the telia commonly coalesce end to end, thus forming long, narrow, dark brown or black lines. On the glumes, these sori occur most commonly, although not exclusively, on the inner surface and are variously grouped. On the kernels, they may be external and evident, variously scattered or grouped, anywhere on the lobes; or they may be internal, that is, within the pericarp in spore pockets after a manner not unlike that noted for the uredinia (fig. 2).

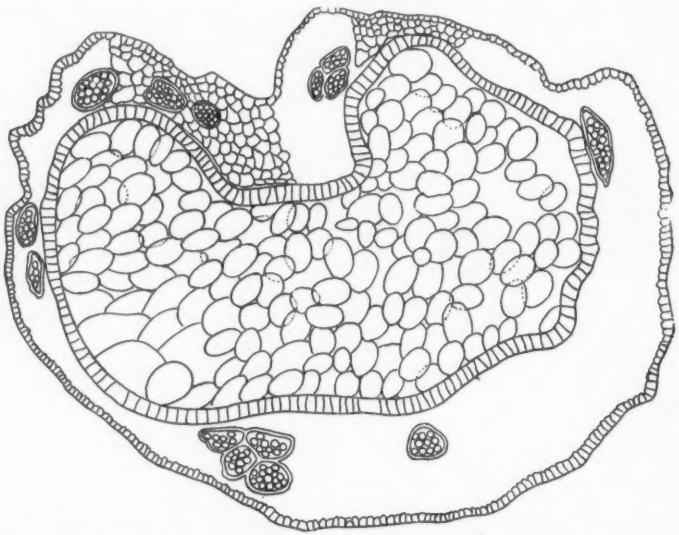


FIG. 2.—Schematic drawing of a cross section of a rust-infected wheat kernel showing pockets or inclusions of rust spores within the pericarp

ON OTHER GRAINS AND GRASSES

The stripe-rust lesions on cereal and grass hosts other than wheat are in most respects similar to those on wheat. It has been observed, however, that there is considerable difference in the color of the uredinia on different grasses. Whether this is due to any actual difference in color of the urediniospores or is traceable solely to depth of spore mass has not been determined. Glume infection has been noted upon *Bromus marginatus* and *B. sitchensis*, but has not yet been observed on any other of the wild-grass hosts.

LIFE HISTORY AND MORPHOLOGY OF THE ORGANISM

Under congenial climatic conditions, the urediniospores of *Puccinia glumarum* retain their viability throughout the winter months and thus constitute an important factor in facilitating the natural spread of infection. Experiments conducted by Eriksson and Henning (*4*) led them to conclude that the germination of the urediniospores was dependent on the influence of low, even freezing temperatures. Particularly did this seem true of spores which had matured early in the season and therefore had been subjected to prolonged exposure to light and desiccation. One of the writers (Hungerford), however, has experienced no difficulty in securing prompt germination of these spores without first subjecting them to subnormal temperatures.

FIG. 3, A.—Schematic drawing of a cross section of a wheat-seedling leaf showing spongy mesophyll and vascular bundles, and absence of sclerenchymatous tissue. B.—Schematic drawing of a cross section of an adult wheat leaf showing vascular bundles, *v*, and supplementary sclerenchyma, *s*. These partitions divide the leaf into as many longitudinal segments of parenchyma

According to Eriksson and Henning (*4*), the urediniospores of *Puccinia glumarum*, like those of *P. graminis*, produce a germ tube of fairly uniform diameter. It is almost invariably unbranched and contains throughout its length the yellow contents of the spore. Where germination takes place on the surface of a living host leaf, there develops at once an appressorium from which is derived the infecting thread or hypha which enters a stoma to proliferate later and form successive masses of mycelium or hymenia which give rise to uredinia and a new crop of urediniospores. Throughout the vegetative period of the host, the disease spreads from leaf to leaf and ultimately echinulate and varies in thickness from about 1 μ to 2 μ. The four to several germ-pores occur irregularly and, except under very favorable optical conditions, are seen with difficulty (fig. 4). According to Eriksson and Henning (*4*), the urediniospores measure from 16 to 19 μ by 25 to 30 μ in diameter. Grove (*6*), on the other hand, gives the dimensions as varying from 18 to 26 μ by 25 to 30 μ. As will be seen from Table I, the writers' figures, based on the measurement of 10,000 spores, show a variation of 12.7 to 25.9 μ by 15.7 to 33.8 μ. The average of 10,000 long and short diameters was 22.63 μ and 18.40 μ, respectively. The wide differences in the two diameters

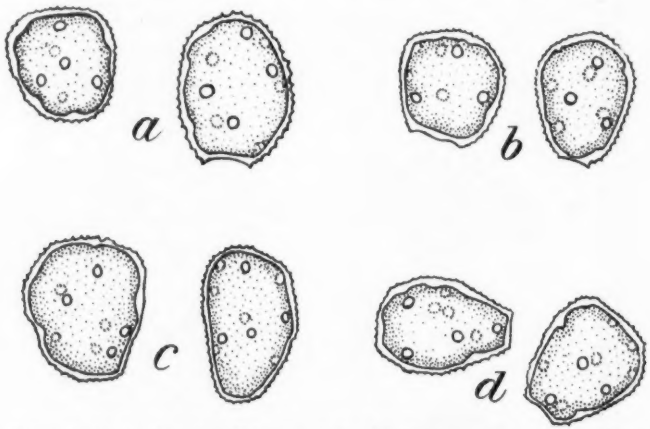


FIG. 4.—Camera lucida drawings of urediniospores of *Puccinia glumarum* on, *a*, *Triticum vulgare*; *b*, *T durum*; *c*, *Hordeum jubatum*; and *d*, *Elymus canadensis*. × 1000

to the entire plant. Under particularly favorable conditions in spring and early summer the amount of initial infection and subsequent development of stripe rust in any one season may be considerable and result in severe damage to the crop.

Marryat (*18*) states that infection resulting from *P. glumarum* obtains, as in the case of *P. graminis*, through entrance of the stomata by the germ tubes of the urediniospores.

UREDINIOSPORES

The urediniospores of *Puccinia glumarum*, sometimes accompanied by hyphoid, incurved paraphyses, vary in form from globose to broadly ellipsoid. The colorless spore wall is moderately

would seem to indicate the possible immaturity of some of the spores. Care was exercised in every instance, however, to confine the measurements to those which were fully mature. Subsequent to the preparation of the data presented in Table I, it was found that the size of the urediniospores apparently is governed to some extent by position on the infected leaf and consequent availability of nutriment. For example, 100 spores from uredinia near the base of a rusted leaf of Baart wheat averaged 18.90 μ by 25.64 μ; a similar number from the middle of the leaf averaged 18.71 μ by 24.84 μ while 100 spores from uredinia at the outer end of the leaf averaged 18.54 μ by 23. 12 μ.

TABLE I.—*Diameter measurements of unrediniospores of Puccinia glumarum*

No.	Host	Collection		Range of size in micra		Average of 200 spores	
		Place	Time	Short diameter	Long diameter	Short diameter	Long diameter
1	*Agropyron cristatum*	Moscow, Idaho	July, 1916	13. 5–24. 2	16. 3–27. 0	18. 33	21. 85
2	do	do	do	14. 5–25. 5	17. 5–29. 6	18. 63	21. 90
3	do	Hilmar, Wash	July 9, 1919	14. 1–23. 0	15. 8–29. 6	18. 07	22. 81
4	*Agropyron cristatum* (Importation from Russia).	Moscow, Idaho	July, 1916	14. 5–23. 9	18. 4–29. 3	19. 27	22. 25
5	*Bromus marginatus*	Pullman, Wash	June 14, 1915	14. 4–21. 8	16. 8–25. 8	18. 04	21. 43
6	*Bromus pacificus*	Friday Harbor, Wash.	June, 1916	15. 3–21. 3	18. 0–28. 3	18. 29	21. 72
7	*Bromus sitchensis*	N. Mt. Vernon, Wash.	do	14. 1–21. 2	15. 4–26. 7	17. 39	20. 66
8	*Elymus canadensis* [a]	Pullman, Wash	July, 1915	14. 7–23. 8	19. 7–33. 8	18. 81	24. 17
9	do	do	July 25, 1915	14. 6–21. 2	18. 1–31. 4	18. 05	23. 722
10	*Elymus condensatus*	Moro, Oreg	Sept. 10, 1916	14. 6–22. 8	18. 5–28. 6	18. 918	22. 817
11	*Elymus glaucus*	N. Mt. Vernon, Wash.	June, 1916	14. 9–24. 2	18. 4–30. 2	19. 02	23. 90
12	do	Friday Harbor, Wash.	do	16. 4–25. 9	16. 7–28. 6	18. 749	22. 071
13	do	do	do	14. 8–24. 1	17. 5–27. 7	17. 867	22. 268
14	*Hordeum jubatum*	Cherry Creek, Denver, Colo.	June 14, 1916	12. 7–21. 0	17. 4–28. 2	17. 294	22. 425
15	do	Denver, Colo	do	14. 2–23. 1	17. 2–27. 9	18. 36	22. 714
16	do [b]	Moro, Oreg	Sept., 1916	13. 7–20. 7	18. 0–27. 7	18. 42	23. 852
17	do	Garfield, Wash	do	14. 2–22. 0	18. 2–27. 9	18. 594	22. 553
18	do	N. Yakima, Wash	June, 1916	15. 3–23. 0	17. 2–30. 0	18. 780	22. 996
19	do	Selah, Wash	June 17, 1916	15. 3–23. 6	18. 6–31. 1	19. 323	23. 830
20	do [c]	Denver, Colo	June 14, 1917	15. 0–23. 8	17. 9 30. 2	20. 135	24. 648
21	*Hordeum murinum*	Los Angeles, Calif	May, 1916	15. 3–21. 7	16. 5–25. 6	18. 748	21. 732
22	do	Del Monte, Calif	do	14. 2–21. 7	16. 7–28. 5	17. 873	22. 537
23	do	Tehachapi, Calif	May, 1915	13. 7–21. 5	18. 4–27. 2	18. 999	22. 475
24	do	Campbell, Calif	May, 1916	15. 2–22. 6	18. 3–20. 3	18. 245	21. 448
25	*Hordeum nodosum*	Mt. Vernon, Wash	Sept. 2, 1916	14. 7–25. 1	17. 6–24. 9	18. 248	21. 128
26	*Hordeum vulgare*	Pullman, Wash	June 14, 1915	14. 4–21. 7	18. 0–29. 8	18. 973	22. 275
27	*Sitanion hystrix*	Redmond, Oreg	July 2, 1914	14. 5–22. 9	18. 0–27. 7	18. 431	22. 859
28	*Sitanion jubatum*	Ashland, Oreg	June 7, 1916	15. 3–23. 4	18. 7–30. 0	18. 462	23. 751
29	do	do	do	14. 2–21. 5	17. 7–29. 2	18. 554	23. 336
30	*Sitanion longifolium*	Red Mountain, Colo	Aug. 26, 1907	13. 7–25. 4	16. 4–29. 2	17. 556	21. 692
31	*Triticum compactum*	Pullman, Wash	Aug. 18, 1915	13. 8–22. 2	17. 5–29. 2	18. 126	21. 976
32	*Triticum dicoccum* (Buffum winter emmer).	Moro, Oreg	June 11, 1915	14. 0–21. 3	17. 7–28. 8	18. 067	22. 776
33	*Triticum durum*	Moscow, Idaho	June 15, 1915	14. 9–20. 4	17. 0–25. 4	17. 504	20. 803
34	*Triticum spelta*	do	July 2, 1919	14. 1–22. 6	16. 2–28. 5	18. 107	21. 303
35	*Triticum vulgare*	Bozeman, Mont	July, 1915	14. 7–23. 2	17. 9–32. 1	18. 245	22. 896
36	*Triticum vulgare* (Chul)	Corvallis, Oreg	June 10, 1915	15. 1–21. 9	19. 1–31. 1	18. 666	24. 172
37	do	do	June 12, 1915	15. 5–24. 6	18. 0–32. 1	19. 154	23. 287
38	*Triticum vulgare*	do	June, 1915	16. 0–23. 0	18. 0–27. 7	18. 721	23. 082
39	do [d]	Bozeman, Mont	July 26, 1915	15. 4–23. 6	19. 0–28. 4	19. 467	24. 181
40	do	Moscow, Idaho	June, 1915	14. 1–21. 3	16. 1–27. 8	17. 727	21. 077
41	do	Spokane Co., Wash	July 20, 1915	12. 7–23. 5	15. 9–27. 8	18. 517	22. 689
42	do	Brigham, Utah	June 23, 1915	14. 4–24. 2	15. 7–31. 1	18. 305	24. 319
43	do	Moscow, Idaho	June 15, 1916	15. 1–23. 3	17. 8–28. 0	18. 102	22. 038
44	do	Casa Blanca, Ariz	May 25, 1915	14. 0–20. 0	17. 9–27. 5	17. 947	22. 219
45	Wheat (species and variety not given).	Moro, Oreg	June 11, 1916	14. 0–23. 5	16. 8–29. 7	18. 322	23. 562
46	do	Freeman, Wash	June 19, 1916	14. 7–22. 4	17. 4–28. 4	18. 471	23. 039
47	Wheat [e]	Bozeman, Mont	Aug. 20, 1915	14. 8–23. 2	19. 0–30. 2	18. 888	24. 272
48	Wheat (Jones Winter Fife).	Pullman, Wash	June 22, 1916	14. 3–23. 3	17. 5–29. 5	17. 382	21, 636
49	Wheat	Eagle, Idaho	June 24, 1916	14. 5–22. 5	17. 8–27. 8	17. 911	22. 574
50	Wheat (mixed)	Mt. Vernon, Wash	June 14, 1916	13. 3–20. 6	17. 9–28. 0	18. 009	21. 705
	Grand average					18. 401	22. 628

[a] Spores very thick-walled, from 0.8 to 3.2 micra.
[b] Nearly all teliospores.
[c] Nearly all teliospores. Shows transition between unrediniospores and teliospores.
[d] Transition from unrediniospores to teliospores.
[e] Telial stage.

Levine (*16*) has shown that although resistant host plants and adverse environmental conditions always tend to dwarf the spores of *Puccinia graminis tritici*, regardless of the physiologic form, this reduction in size is not permanent. He has shown that when cultures from such spores are maintained under normal growth conditions the resultant spores are of characteristic size and shape. The same condition also doubtless holds for the spores of *P. glumarum*.

TELIOSPORES

The teliospores of *Puccinia glumarum* develop in hypophyllous or culmicolous sori. They occur in long, narrow lines; and in those wheat varieties where glume infection is common, form oblong blackish patches, in all cases covered by the epidermis. The spores, commonly 2-celled, are clavate to rounded or obliquely conical above, with smooth exospore which varies in thickness from about 1 μ to 3–6 μ for the thickened apex (fig. 5). They are brown, somewhat constricted, attenuate at the base, and measure, according to Eriksson and Henning (*4*), 16 to 24 μ wide by 30 to 40 μ long. In his description of the teliospores Grove (*6*) gives a width varying from 12 to 24 μ and a length of 30 to 70 μ. The writers' figures, derived from the measurement of thousands of mature teliospores, approach more nearly those of Grove, and vary from 12 to 27 μ by 30 to 65 μ. The spores, borne on pale, short and usually persistent pedicels, occur in groups surrounded by long sterile curved cells or paraphyses. According to Arthur (*1, p. 338–339*) the teliospores germinate at maturity.

Seed infection of wheat by *Puccinia glumarum*, which was first reported by Eriksson and Henning (*4*) and later by Blaringhem (*3*), has been noted by the writers most commonly in certain varieties, particularly Chul. The infection occurs in the pericarp of the wheat kernel, where it develops as mycelial pockets containing either urediniospores or teliospores or both (fig. 2).

This seed infection suggests potential importance from the standpoint of seed transmission, which problem is being investigated by one of the writers (Hungerford) and the results will be published separately.

THE FUNGUS IN RELATION TO DEVELOPMENT OF THE DISEASE

SEASONAL OCCURRENCE

As is true of other cereal rusts, stripe rust reacts to the conditions imposed upon it by temperature, relative humidity, soil, and other factors affecting the growth of both parasite and host. The absence of an alternate host and the relatively short period of viability of the urediniospores make more or less fortuitous the occurrence of general and destructive outbreaks of this rust. Given a source of inoculum of sufficiently wide distribution, accompanied by a combination of high relative humidity, relatively low fall temperatures, and complete susceptibility of host, a general and severe outbreak of seedling infection may develop during the fall. If the crop is sufficiently protected from alternate freezing and thawing during the winter to prevent winter killing, the mycelium of the rust fungus will hibernate and be present as a potential factor for the development of new urediniospores the following spring.

From the observational data in hand it is apparent that the further development and spread of stripe rust during the spring depend primarily on continuous high humidity and relatively low temperatures. On the other hand, it is true that a much more critical examination of meteorologic data and of other information bearing a possible relation to infection and spread of stripe rust in both the United States and Europe will need to be made before it can be stated very certainly what combination of conditions most definitely determines the seasonal occurrence and severity of stripe rust.

VIABILITY AND LONGEVITY OF SPORES

The literature extant on stripe rust contains but scant information on the duration of viability of the spores of *Puccinia glumarum*. Investigators, studying the problem of seasonal propagation of the rust, have emphasized the importance of hibernating mycelium, and have shown that even in the far northern countries of Europe, stripe rust may, and frequently does, survive the vicissitudes of winter as viable mycelium within the tissues of the host

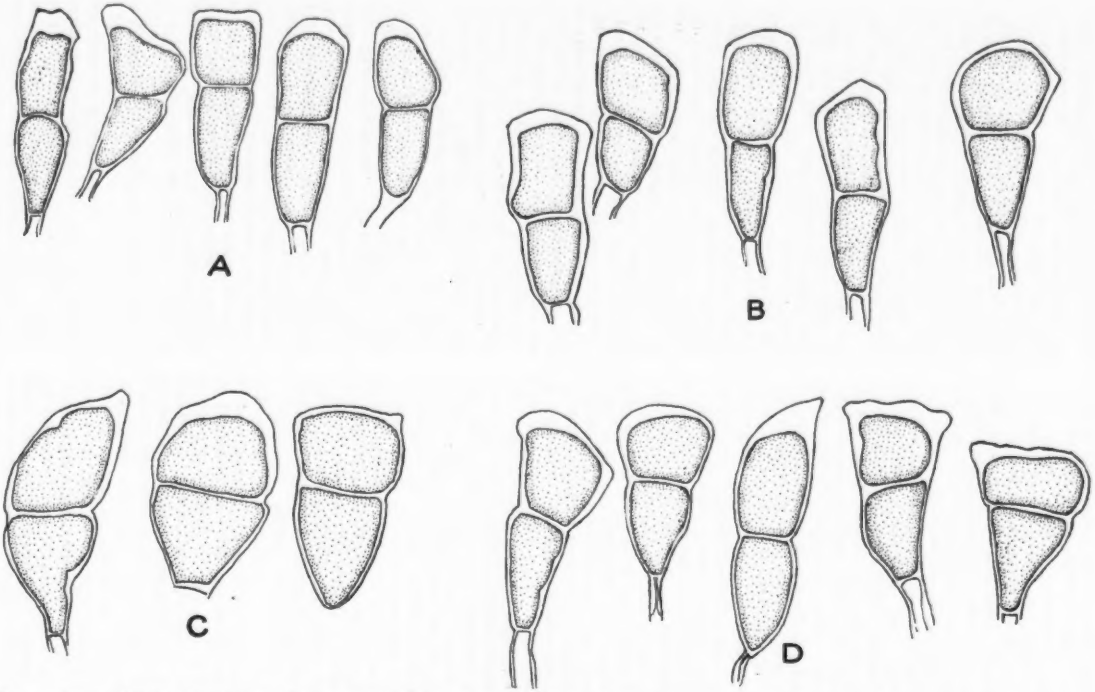


FIG. 5.—Camera lucida drawings of teliospores of *Puccinia glumarum* on, *A, Triticum compactum; B, Hordeum jubatum; C, Elymus striatus; D, Triticum vulgare*, showing variation in size and form of spores taken at random. × 1000

plant. Aside from the teliospore germination studies conducted by Eriksson and Henning (*4*) in the early 90's, but little further work has been done to determine either longevity or possible function of the teliospores of this rust. The investigation of this phase of the stripe-rust problem by one of the writers (Hungerford) is now in progress at Moscow, Idaho.

SOURCES OF NATURAL INFECTION

Until the two recognized races of *Puccinia glumarum* in North America have been critically studied in the light of their differential host relationships, knowledge of possible sources of natural infection will be but fragmentary and more or less uncertain. According to Hungerford (*11*), stripe rust already has been found to occur naturally in this country on wheat, barley, and rye, and 39 wild grasses. Two grasses very commonly infected are *Hordeum jubatum* and *Bromus marginatus;* and the form of the rust occurring on these hosts goes readily to wheat. *Hordeum jubatum* is common throughout the Northern States from Washington to Maine, yet the rust has not traveled eastward beyond 103° W. longitude. It probably is true, however, that the wild-grass hosts play an important rôle in the spread of stripe-rust inoculum in the form of urediniospores that have survived the winter and new urediniospores which have been produced by hibernated mycelium. Irrespective of any wild-grass hosts, there is always the possible winter survival of both urediniospores and mycelium in the infected tissues of the cultivated hosts, wherever winter cereals are grown. Volunteer wheat, barley, and rye, developing as they usually do very early after the first late summer rains, frequently become infected and serve as sources of inoculum for the infection of the fall-seeded crop.

Hungerford (*10*) conducted extensive studies to determine the possible relation of wheat kernels infected with *Puccinia graminis tritici* to the occurrence of initial infection in seedlings grown from such kernels. His evidence was wholly negative. The same author (*11*) obtained like results in his investigation of the problem of seed transmission of living mycelium and urediniospores of *P. glumarum*.

Eriksson and Henning (*4*) assert that it may be accepted as an established fact that this rust frequently lives from one crop season to the next in a dormant or resting-mycelium stage, present in infected seedlings of winter wheat. They do not show, however, that such winter survival of the fungus is necessary to its perpetuation, even in the far northern agricultural districts of Sweden. Indeed, their experimental study of the relation of such factors as humidity, light, and temperature to germination of the urediniospores would indicate that low temperatures enhance rather than inhibit their germination. They do not deny the fact that hibernating urediniospores, under favorable conditions, may assist materially in accelerating a severe epidemic of rust; but it is their opinion that the severity and extent of such an epidemic are more directly dependent on dormant mycelium.

Biffen (*2*) states that stripe rust is the most destructive of the wheat rusts common to England and that winter survival of *P. glumarum* under the conditions of England's winter climate is very largely due to viable urediniospores which have survived the winter.

Mehta (*19*), in his comparative study of the viability and germination of the spores of cereal rusts, found the urediniospores of *P. glumarum* to be short-lived and their vitality impaired by temperatures easily withstood by the urediniospores of *P. graminis* and *P. triticina*. His experiments indicated that the urediniospores of *P. glumarum*, exposed to temperatures as low as 2.5° to 5° C., retained their viability less tenaciously than do those of *P. triticina*. He found that 15 to 20 per cent of them survived an exposure of a month at the above temperatures.

The writers' observations on the probable overwintering of living mycelium agree with those of Eriksson and Henning. In connection with an experiment conducted by one of the writers (Hungerford), designed to determine the possibility of seed transmission of the disease, three lots of Chul wheat were sown in October, 1916. One lot of seed was badly infected with stripe rust. Another lot, similarly infected, had been given the modified hot-water treatment; and the third lot was of clean, rust-free seed. Somewhat later, through a misunderstanding, some plants of *Bromus marginatus* and *Hordeum jubatum* infected with *P. glumarum* were set out adjacent to the above plats of wheat. During the winter, both grasses died down to the ground while the wheat remained green. On April 25, 1917, the rust was found on three or four wheat plants immediately next to these grasses. Subsequently, the rust spread rapidly from this center of infection

and with the development of new leaves the grass plants also became heavily infected. From the foregoing, it would appear that viable urediniospores from one or both of the grasses had found lodgment on the leaves of the nearby wheat plants, and that the subsequent infection of the latter was traceable to spores produced in the grass hosts.

Rostrup (*20, p. 260*) is of the opinion that the stripe-rust fungus hibernates as living mycelium, and that on resumption of growth is followed promptly by the development of uredinia. Apparently the temperature permitting such renewed activity is relatively low, for, as pointed out by Henning and Bygdén (*9*), stripe rust not uncommonly appears shortly following the melting of the snow. In Denmark it was more or less prevalent in April of 1906, 1910, 1911, and 1914. In 1910 a new crop of urediniospores sufficiently mature to germinate was found as early as the first week of March.

Klebahn (*14*) tried to show by experiment the hibernating capacity of the urediniospores of *Puccinia glumarum*, but obtained only negative results. He was successful, however, in securing germination of urediniospores of *P. dispersa* Erikss. and Henn. that had survived the winter. Hecke (*7, 8*) studied the same problem in Austria and showed that the mycelium of *P. glumarum* could endure a temperature of −10° C., or lower. He further pointed out what is probably a fact, namely, that the mycelium of this rust organism will survive such winter conditions as may not prove too rigorous for the host.

K. Murashkinski, Director of the Western Siberian Laboratory of the Siberian Agricultural Academy, Omsk, Siberia, in correspondence with the senior writer, states that he had locally observed *Puccinia glumarum* on *Bromus unioloides* H. B. K. and *Elymus dahuricus* Turcz. in 1920 and 1921, and that infection seemed to be confined solely to these two grasses. Omsk is situated in latitude 55° N. and is subject to extremely low winter temperatures. The moderately heavy and continuous snow cover of that region from October until April, however, undoubtedly affords ample protection to both host and parasite, thus insuring the propagation of the rust organism by means of hibernating mycelium.

CLIMATE IN RELATION TO DEVELOPMENT OF STRIPE RUST

Unpublished meteorologic data furnished by D. E. Stephens,[4] for the period September, 1914, to September, 1915, inclusive, would seem to indicate beyond doubt the nice dependence of *Puccinia glumarum* on climatic conditions. During May and June, 1915, stripe rust developed at the substation to the point of a genuine epidemic. Given the necessary inoculum to insure a general distribution of seedling infection during September and October, 1914, the heavy and almost continuous snow cover of the winter of 1914–15 furnished ideal overwintering conditions. Reference to Table II will show how favorable for infection were the precipitation and temperature conditions of September and October, 1914. It will be noted also that these same factors during the spring and early summer months of 1915 were such as to promote the further development and spread of the rust. If these data are compared with those for September and October, 1915, and those of the spring and early summer months of 1916, it will be noted that the factors favoring early emergence and normal subsequent fall growth of volunteer wheat were quite as ideal during September and October, 1915, as they were during the same period in 1914. Moreover, assuming that the 1915 epidemic had its origin in hibernating mycelium arising from infection during the autumn of 1914, the amount of available rust inoculum (urediniospores) must have been very much more abundant than in the fall of 1914. It also should be noted that the winter of 1915–16 was characterized by a nearly continuous snow cover. The temperature and moisture conditions of March, April, and May, 1916, were not inimical to the development of stripe rust, yet 1916 was not a rust year. Commercial wheat fields inspected in June showed but a trace and only the more susceptible varieties growing on the station farm were infected.

[4] STEPHENS, D. E.—REPORT OF THE SHERMAN COUNTY BRANCH STATION, MORO, OREGON, 1914–16. [Unpublished. Copies in Office of Cereal Investigations, U. S. Dept. Agr., Washington, D. C.] (C. E. HILL, joint author, 1915–16.)

TABLE II.—*Meteorologic data for the period January to December, 1914, 1915, and 1916, inclusive, recorded at the Sherman County Branch Station, Moro, Oreg.*

Month	Air temperatures (°F.)									Precipitation (inches)			Evaporation (inches)		
	Maximum			Minimum			Mean								
	1914	1915	1916	1914	1915	1916	1914	1915	1916	1914	1915	1916	1914	1915	1916
January	43	41	20	30	10	10	36	26.0	15.0	2.20	1.75	1.10	.....	.....	.....
February	39	52	35	27	23	26	33	36.0	30.5	1.16	2.31	2.43	.....	.....	.....
March	54	70	50	34	30	35	44	45.0	42.5	.11	1.27	2.05	.....	.....	.....
April	59	75	59	39	32	37	49	50.8	48.0	2.06	.65	.75	4.01	5.23	4.80
May	70	78	61	42	34	39	56	53.0	50.0	.76	2.06	1.37	7.43	5.73	5.68
June	72	93	72	47	40	47	59	60.0	59.5	.66	.36	1.98	8.29	8.24	6.80
July	85	96	74	56	40	51	70	64.8	62.5	.08	.57	.92	11.43	8.95	7.88
August	85	100	82	54	46	54	69	71.3	68.0	Trace	.05	.15	9.64	9.61	7.50
September	67	86	72	44	36	45	55	57.3	58.5	1.05	1.14	.33	4.40	5.23	4.68
October	59	73	62	40	33	34	49	50.6	48.0	1.48	.23	.39	2.19	2.93	2.81
November	47	54	41	30	25	26	38	38.2	33.5	.88	2.89	1.69	.....	.45	.....
December	28	53	35	17	11	23	23	32.8	29.0	.88	1.61	1.32	.....	.....	.....

According to Lang (*15*), who has made a careful investigation of climatic factors in relation to the occurrence of stripe rust in Europe, the following comprise the conditions typical of a severe epidemic: An abundance of hibernating mycelium, followed by the development of uredinia in late March or early April. This first crop of urediniospores, favored by congenial conditions for germination and incubation, will give rise to a second, and from the second a third crop of spores. Thus, by early May, assuming conditions to be ideal, a severe and general epidemic will have developed. Spring wheat sown in April will become infected by the middle of May, and by the end of the month the second principal outbreak of the rust will have occurred.

Litvinov (*17*), commenting on the probable causes which contributed to the severe epidemic of stripe rust in central Russia in the summer of 1914, attributes the attack to favorable climatic factors. The spring of 1914 was relatively cool, but free from recurrent thaws. The general coolness of the spring contributed to the early development of urediniospores as contrasted with *Puccinia triticina*. His observations were confined to experiments with varieties of spring wheat, in which he noted that susceptible early varieties were much more subject to damaging infection than were late-sown varieties of approximately equal susceptibility. Jenkin and Sampson (*13*) in Wales likewise noted a correlation between date of seeding and severity of infection, but their results indicate that the severest infection develops on the latest-sown rather than on the earliest-sown plats. It is possible that this difference in correlation may be traced to differences in contingent climatic conditions which characterized the spring of 1914 at Voronezh and that of 1920 at Aberystwyth.

Although the foregoing instances of severe, even damaging, infection in spring wheat would seem to controvert the evidence adduced by the majority of the students of stripe rust to the effect that serious infection seldom develops in spring wheats, it should be pointed out that even such instances may be concerned with epidemics originating from mycelium which may have hibernated in some near-by grass host or in volunteer wheat plants.

Thus it is conceivable that climatic conditions which favor a general seedling infection during the autumn months may contribute to the development of a high potential for a subsequent and destructive epidemic during the spring and summer. But it is apparent that unless the fungus is favored by optimum spring conditions it will not develop to economic proportions.

It is obvious from the foregoing that until a more extended and critical study has been made of all possible factors influencing infection by, and subsequent development of, the stripe-rust fungus, it will not be possible to answer the many perplexing questions that must arise in the mind of the investigator.

CONTROL OF STRIPE RUST

The adoption of varieties of wheat resistant to stripe rust is the most practical means of control. Jenkin and Sampson (*13*) found that of the more than 90 varieties and strains of wheat

included in their experimental study of varietal resistance to both *Puccinia glumarum* and *P. graminis*, Garton's Early Cone and Percival's Blue Cone proved resistant to both rusts. They found that certain varieties, seemingly rust-resistant, were merely rust-escaping because of their more rapid growth and maturity. The general adoption of such spring wheats undoubtedly would serve to reduce the danger of loss from stripe rust, but it is hardly probable that this would apply to winter varieties for the reason that the occasional presence of hibernating mycelium would make possible early and destructive outbreaks of stripe rust.

As in Europe, so also in the United States, wheat varieties differ markedly in their susceptibility to stripe rust. Moreover, varieties differ in susceptibility to infection both in the heads and in the leaves. Observations made by writers in 1915 at Corvallis, Oreg., on Chul wheat showed this variety heavily infected in the floral parts, especially the glumes, while but slight infection was noted on the blades and sheaths. Kernels from these infected heads were later examined microscopically and found to contain abundant internal infections. A number of other wheat varieties growing alongside showed no infection on the floral parts but a considerable amount of it on the leaves. One of the writers (Hungerford) has made similar observations. An excerpt from a summary of notes taken by him on an inspection trip made in 1916 presents the following interesting data on the subject of varietal resistance to stripe rust: "There seemed to be not only a vast amount of difference in the susceptibility of the various varieties of wheat to stripe rust but also there was a difference in the amount of infection appearing upon the glumes and on the kernels. Chul wheat did not seem to be especially heavily infected on the leaves, but was, in every case where noted, infected in the glumes to a marked degree."

During the first year (1915) stripe rust was observed in the United States, marked varietal differences in susceptibility to leaf infection were noted.[5] In a series of wheat varieties growing in adjacent twentieth-acre plats at Moro, Oreg., the leaves of Dale (C. I. No. 4155) were the most heavily infected, averaging about 85 per cent infection. Hybrid 123 (C. I. No. 4511), Baart (C. I. No. 1697), Little Club (C. I. No. 4066), Purplestraw (C. I. No. 1915), Turkey selection (C. I. No. 2998–1), and Beloglina (C. I. No. 2239) also were rather heavily infected, the infection ranging from about 75 per cent to 40 per cent in the order given. The other wheat varieties in the series all showed infections varying from about 30 per cent to a mere trace. During the same year (1915) Little Club showed about 65 per cent of infection at Moscow, Idaho, while other varieties there showed only slight infections or none at all. Subsequent observations and experiments by Hungerford and Owens (*12*) have shown more fully the relative susceptibility of different wheat varieties to stripe rust.

SUMMARY

(1) Collections of *Puccinia glumarum* were made in North America by various American botanists during the 90's and the early years of the present century. These collections were distributed under names other than *P. glumarum*.

(2) *Puccinia glumarum* was found and recognized in the United States by the late F. Kølpin Ravn of Copenhagen, Denmark, in May, 1915.

(3) The common name, stripe rust, is proposed in lieu of yellow rust or golden rust as a common name for the disease caused by *Puccinia glumarum*. This accords with the Italian name, *ruggine striata del grano*.

(4) *Puccinia glumarum* is now known to occur from British Columbia to Mexico and eastward to 103° W. longitude. It has been found in all of the Pacific and Intermountain States except Nevada and New Mexico.

(5) Stripe rust is a disease of considerable economic importance in Great Britain, Northern and Central Europe, North Africa, Japan, and India. Its destructiveness in the United States would undoubtedly be fully as serious as that experienced in "rust years" in Europe should it become established in the soft red winter-wheat areas east of the Mississippi River.

(6) *Puccinia glumarum* is now known to occur in nature on 34 wild grasses common to the United States in addition to the cultivated hosts, wheat, barley, rye, spelt, and emmer.

(7) Certain varieties of wheat apparently are much more subject to glume and kernel infection than others. All susceptible varieties show general leaf infection.

(8) An aecial host for *Puccinia glumarum* has not yet been discovered.

(9) The dimensions, particularly the length, of urediniospores produced on the leaves vary according to location on the leaf. Those occurring in ure-

[5] These early observation were made jointly by two of the writers (Humphrey and Johnson) and Dr. F. Kølpin Ravn.

dinia nearest the base of a given leaf are larger than those from uredinia located near the distal end.

(10) Infection of most hosts and varieties is confined to the leaves and culms.

(11) The uredinia and telia of *Puccinia glumarum* not infrequently occur on the kernels and glumes of certain varieties of wheat. Such infection usually causes noticeable shrinkage of the kernels and apparently affects their viability.

(12) The severity and spread of stripe rust in any given locality devoted to winter wheat depend primarily on (*a*) fall weather conditions favorable to seedling infection; (*b*) successful hibernation of mycelium; (*c*) spring weather conditions favorable to germination of urediniospores.

(13) *Puccinia glumarum* in the Pacific Coast States has been found capable of winter survival by means of living mycelium or viable urediniospores.

(14) Varietal-resistance studies indicate the existence of several varieties of wheat which are highly resistant to *Puccinia glumarum*. Among the most resistant are Turkey (C. I. No. 1558), Turkey (C. I. No. 1750), Alton (C. I. No. 1438), Prohibition (C. I. No. 4068), and Red Russian (C. I. No. 4222).

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A STUDY OF BACTERIAL PUSTULE OF SOYBEAN, AND A COMPARISON OF BACT. PHASEOLI SOJENSE HEDGES WITH BACT. PHASEOLI EFS.[1]

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INTRODUCTION

In the fall of 1916 badly spotted soybean leaves were received from Texas. The leaves were thickly covered with small, brown, angular, slightly raised spots (Pl. 2 A), from which a yellow organism resembling *Bacterium phaseoli* EFS. was isolated, and inoculations with this produced the disease on healthy soybean plants. The same disease was observed by Erwin F. Smith in 1902 on leaves from Charleston, S. C., and by the writer in the Dismal Swamp, Virginia, in 1917. In both cases an organism like *Bacterium phaseoli* was isolated. In later years it has been received from other localities.

HISTORY OF THE DISEASE

The first mention in literature of this disease is a brief note by Smith(*17*)[2] in 1904; and the leafspot referred to by the same author in "Bacteria in Relation to Plant Diseases" (*18, v. 1, p. 92; v. 2, p. 69*) is the one here under discussion (oral communication), but no description of the disease or organism was given. Heald (*6, p. 41, 71*) in 1905 and again in 1906 (*7*) described very briefly a bacterial blight of soybean, but no cultural studies were made and it is impossible to tell with certainty whether the disease which came under his observation was the one discussed here or the leafspot described by Johnson and Coerper (*10, 4*), resembling the pustule disease in certain stages but caused by a white organism, *Bact. glycineum* Coerper, or whether it was the one attributed by Wolf (*22*) and Shunk (*14*) to *Bact. sojae* Wolf, which resembles *Bact. glycineum* so closely that it is questionable whether one or two diseases are involved.

Clinton (*3, p. 444–445*) in 1915 gave a more detailed description of a bacterial leafspot of soybean and considered it identical with the bacterial disease of wax and Lima beans and due to *Bact. phaseoli* EFS., but made no cultural studies. Bryce (*2*) in 1917 reported the occurrence in Canada of a bacterial blight of soybean leaves, but accompanied this statement with no description; and the Plant Disease Survey of the United States Department of Agriculture (*5, p. 116*) recorded a bacterial leafspot on soybean in Pennsylvania and Indiana in 1918, with no clue as to which bacterial disease was meant.

Three other species of bacteria are known to be infectious to soybean in the United States: *Bacillus lathyri* Manns and Taubenhaus (*11, p. 12; 21, p. 141*), *Bact. solanacearum* EFS. (*19*), and *Bact. flaccumfaciens* Hedges(*8*), but there is no danger of confusing any of the diseases caused by these organisms with the one under discussion.

In 1902, Smith isolated from soybean leaves the yellow bacterium resembling *Bact. phaseoli* EFS., and reproduced the disease on healthy plants by pure culture inoculations. Infections resembling those caused by *Bact. phaseoli* EFS. were also produced on varieties of *Phaseolus* sp. But, although several attempts were made, no infection with very virulent colonies of *Bact. phaseoli* EFS. isolated from Phaseolus was ever obtained on soybean by Smith (oral communication). Thus the question as to the identity of the two parasites has long been an open one, and in an attempt to solve this problem the writer undertook the present work, a preliminary account of which has been published (*9*).

GEOGRAPHICAL DISTRIBUTION

Little is known in regard to the geographical distribution of this disease. The writer has received material from Texas (1916), Virginia (1917 and

[1] Received for publication Apr. 19, 1924—issued January, 1925.
[2] Reference is made by number (italic) to "Literature cited," p. 250–252.

1921), Arkansas (diseased seed, 1919), Louisiana (1922), Kansas (1923, 1924), and Delaware (1924) only, but as Smith obtained it in 1902 from South Carolina, it is quite probable that some, at least, of the "bacterial leafspot" of soybean reported from time to time by various investigators has been due to the organism herein described.

This organism or *Bact. glycineum* was present in South Dakota in 1922, but the material sent in was very dry and attempts at isolation of the parasite failed.

SIGNS OF THE DISEASE

The disease is characterized by angular reddish-brown spots on the leaves, varying in size from minute inconspicuous specks to large irregular brown areas involving a considerable portion of the leaf (Pl. 1, A to G). The latter are caused by a fusion of the smaller spots and are not uniform in color, but mottled in appearance. The original small spots can usually be distinguished in these larger brown areas caused by their fusion. Portions of the large spots frequently drop out, leaving the leaf ragged. The browning sometimes follows the veins (Pl. 2, B and C).

A common and conspicuous, though not universal, sign of the disease is a pronounced yellowing. When present, it occurs as a yellow border around each individual spot or as a yellowed area thickly beset with tiny brown spots or inclosing larger brown areas formed by the expansion and fusion of the same (Pl. 1, A to G). This yellow color varies from a pale yellowish green to a light cadmium (*13*).

The first signs of the disease are very small, inconspicuous, pale green or sometimes reddish-brown spots (Pl. 1, D), usually slightly but distinctly elevated in the center. Sometimes the whole spot is elevated, making a minute pustule (Pl. 1, F), sometimes only the central portion, which may be lighter colored than the pale yellowish-green periphery or may be reddish brown. These intumescences may be confined to either surface of the leaf or may extend through it and appear on both sides. They finally rupture the epidermis but rarely if ever attain a size of more than 1 mm. in diameter, and as the spot grows older they usually collapse, shrivel, or slough off. They are truly tumorlike to this extent, that they are due to both hypertrophy and hyperplasia (Pl. 3, A, C, D). *At no stage is there a water-soaked appearance of the tissues such as is so striking in the early stages of the blight caused by Bact. glycineum Coerper.*

In August, 1923, at the experimental farm of the University of Wisconsin the writer observed on many varieties of soybeans a heavy infection of *Bact. glycineum* and confirmed a previous belief that when young infections are present the two diseases are easily distinguishable, but at a later stage only the isolation of the parasite can insure a correct diagnosis.

The writer has observed infection of the pods only once, viz, in some greenhouse experiments on the Hahto soybean, the pods of which are less hairy than those of the other varieties used up to that time; but in all probability it occurs frequently under favorable field conditions. The spots were brown and slightly raised and the organism was isolated therefrom.

MICROSCOPIC APPEARANCE

Microtome sections through the pustules on Hahto soybeans three weeks after inoculation show that the bacteria (stained with carbol fuchsin)

EXPLANATORY LEGEND FOR PLATE 1

Natural infections and greenhouse inoculations. All natural size except F. Paintings by James F. Brewer.

A to I, *Bact. phaseoli sojense* Hedges:

A.—Natural infection on Mammoth Yellow soybean. Collected at Arlington, Va., October 7, 1921.

B.—Natural infection on Tarheel Black soybean. Collected at Arlington, Va., October 7, 1921.

C.—Natural infection on Barchet soybean. Collected at Arlington, Va., October 7, 1921.

D.—Natural infection on Mammoth Yellow soybean. Very early stage which might easily be overlooked. Collected at Arlington, Va., October 7, 1921.

E.—Artificial infection on Hahto soybean, inoculated March 11, 1919. Painted April 3, 1919; 3 weeks old. Pustule stage.

F.—Detail from Figure E showing pustule. ×10 ca.

G.—Artificial infection on Wilson Black soybean, inoculated March 12, 1920. Painted May 28, 1920; 6 weeks old.

H.—Bush string bean leaf sprayed with colony 6, October 20, 1919 (through bush string bean) January 19, 1920. Painted February 9, 1920; 3 weeks old.

I.—Bush string bean pod; inoculated March 1, 1920, with colony 6, October 20, 1919 (through bush string bean). Painted March 22, 1920; 3 weeks old.

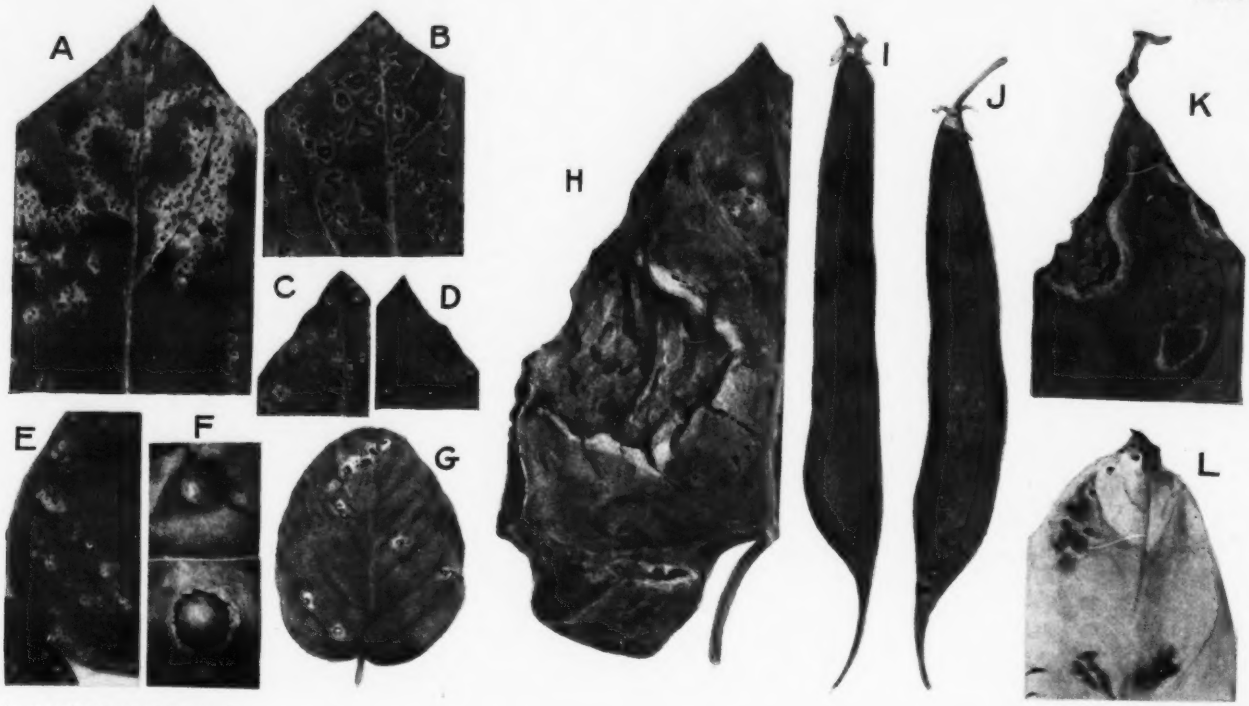
J to L, *Bacterium phaseoli* E F S.:

J.—Secondary infection on bush string bean pod on plant inoculated January 2, 1920 (in blossom at the time). Painted February 26, 1920.

K.—Bush string bean leaf, inoculated January 2, 1920. Painted February 7, 1919, 5 weeks old.

L.—Bush string bean leaf, inoculated January 2, 1920. Painted February 7, 1919, 5 weeks old; showing only islands of green.

Washington, D. C.


A
B
C
D
E
F
G
H
I
J
K
L

are present in abundance, and that the swelling is caused chiefly by hypertrophy and hyperplasia of the parenchyma (Pl. 3, A, C). In some cases there was also striking hypertrophy of the epidermis cells (Pl. 3, D), but no hyperplasia was observed in this layer. The bacteria occur throughout the intumescence and there are bacterial cavities occupied by masses of bacteria, also innumerable areas where individual rods can be clearly seen (Pl. 3, B).

The tissues of a pustule were tested with KI and H_2SO_4 for cork seven weeks after inoculation. The cell walls gave the yellowish-brown reaction indicating suberized tissue, although none of the cells resembled cork cells in appearance. In fact, only a very small portion of the cell walls in the diseased area, whether in the swollen portion or not, showed the blue cellulose reaction.

ISOLATION OF THE PARASITE

The parasite is easily isolated from fresh material by means of agar poured plates. In some cases there was no previous sterilization of the leaf surface, but in the majority of cases $HgCl_2$ 1:1000 was used, the leaf being dipped first in alcohol, and subsequently thoroughly rinsed in sterile water to remove the $HgCl_2$. It was found that 10 to 20 seconds was a good average time for sterilization. The tissue was then crushed in a tube of sterile water or beef bouillon with a sterile rod and diffusion was allowed to take place for 10 to 20 minutes, or longer if the tissues were dry or microscopical examination had shown comparatively few bacteria present. The writer has safely used distilled water for rinsing the leaves and preparation of the dilutions, although this procedure has caused trouble with certain more sensitive organisms. The colonies are usually visible to the naked eye in 2 to 3 days but occasionally fail to appear until 4 or 5 days have passed.

INOCULATIONS ON SOYBEAN WITH BACTERIUM PHASEOLI SOJENSE

The disease has been reproduced repeatedly on sound soybean plants in the hothouse both by spraying and by rubbing with absorbent cotton wet in a water suspension of a culture of *Bact. phaseoli sojense* (Pl. 1, E to G; Pl. 2, B to D). The best results were obtained when young rapidly growing plants were used and were kept moist for at least 36 hours after inoculation. This was done by placing them in inoculating cages, the interior of which was thoroughly wet previous to inoculation. The cages were covered with heavy Manila paper and the plants were kept moist by repeated sprayings with bacterial suspensions during the 36 to 72 hours.[3] If very small plants were used and the only available inoculation cage was large, pans of water or large plants with plenty of transpiring surface were added.

Good infections were also obtained when the plants were covered with paraffined bags, but these did not hold the moisture as well as the cages and it was more difficult to respray the plants. Placing the sprayed or rubbed plants under a bench in the shade was sometimes sufficient.

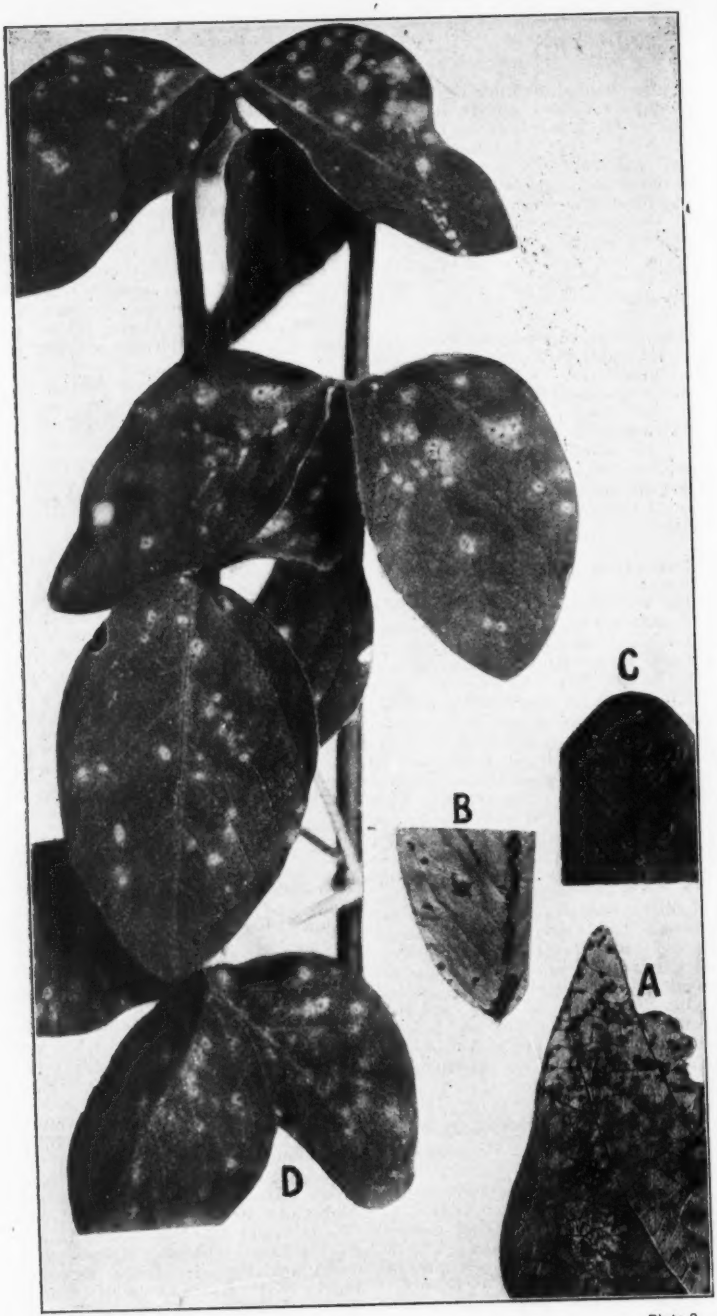
The first signs of infection usually appeared in 5 to 9 days, although in some cases there was a longer incubation period. Generally, the pustules were plainly visible in less than 10 days and the infection progressed as described under "Signs of the disease," above. Under greenhouse conditions the leaves rarely were as badly diseased as in the field. This was probably due to the fact that the soybean plants themselves did not thrive particularly well in the conditions prevailing in the Washington hothouses.

SUSCEPTIBLE VARIETIES OF SOYBEANS

Successful inoculations were made upon the following varieties of soybeans: Ito San, Medium Yellow, Mammoth Yellow, Hahto, Wilson Black, Hollybrook Early. No infection was obtained on Wilson Fine, although plants of Ito San and Medium Yellow of the same age (3 weeks) inoculated at the same time with the same culture became badly diseased.

Natural infection was observed in the fall of 1921 on the following varieties at Arlington Farm, Va.: Auburn, Austin, Barchet (Pl. 1, C), Biloxi, Buckshot, Chiquita, Edna, Fairchild, Flat King, Hahto, Hinangdon, Hollybrook, Honkong, Hoosier, Laredo, Mammoth Brown, Mammoth Yellow (Pl. 1, A and D), Manchuria, Mandarin, Midwest, Mikado, Mixture, Tarheel Black (Pl. 1, B), Tokio, Wisconsin Black, S. P. I. 49832,[4] 49918, 51042, 49834, 51043 (new introductions by S. P. I. with no varietal names), S. P. I.

[3] The plants were never kept in the cages as long as 72 hours when the temperature was high.
[4] Office of Seed and Plant Introduction, U. S. Dept. of Agriculture.



Bacterial Pustule of Soybean

Plate 2

Bacterium phaseoli sojense on soybean. (Photographs by JAMES F. BREWER)

A.—Natural infection, Texas. ×1
B.—Artificial infection 6 weeks old, with colony through Lima bean. ×1½
C.—Artificial infection 7 weeks old, with colony directly from soybean. ×1
D.—Soybean 4 weeks after spraying with colony passed through Lima bean. ×1½

49920, 50440, 50441, 51045, 51046, 52345, 52346, 52347, 52348, 52349 (Chinese varieties introduced in 1920–21).

Midwest was a particularly susceptible variety. There were few, if any, sound leaves. Hollybrook, Mikado, Tokio, Mammoth Brown, Hahto, Tarheel Black (Pl. 1, B) and some of the Mammoth Yellows (Pl. 1, A) were also badly infected. Buckshot was so slightly diseased that some search was required to find infected leaves. Mandarin and Biloxi also had a very small percentage of infection.

INOCULATIONS ON PHASEOLUS WITH BACTERIUM PHASEOLI SOJENSE

In an effort to determine whether the soybean organism was identical with *Bact. phaseoli* EFS., many cross inoculations were made. All inoculation experiments were carried on in the greenhouse. Infections were obtained on the leaves and pods of a number of the members of the genus Phaseolus with the soybean organism (Pl. 1, H and I; Pl. 4, A to C, E and F). On the other hand, repeated attempts to infect the soybean with *Bact. phaseoli* EFS. met with failure until inoculation was made under very abnormal conditions described later.

Successful inoculations were made with the soybean organism on Climbing Lima, King of the Garden Lima, bush string bean, dwarf bush string bean, dwarf bountiful string bean and dwarf wax bean.

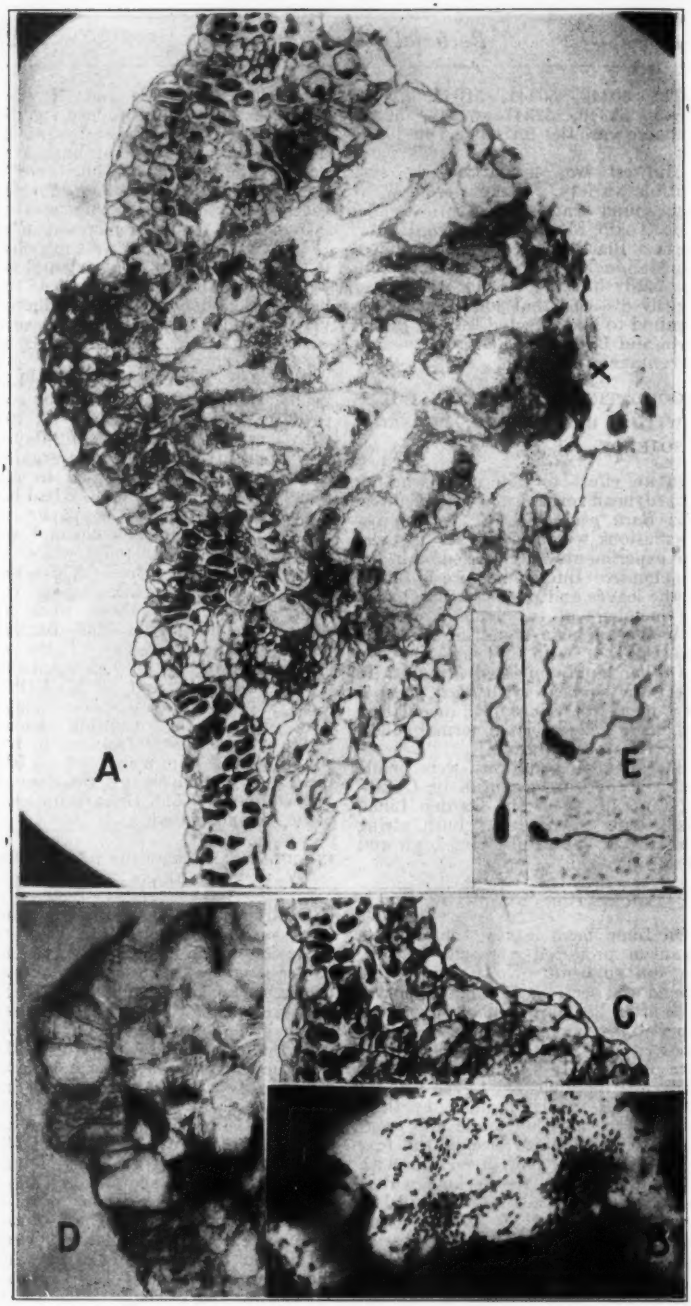
INOCULATIONS ON LIMA BEAN

On Lima bean leaves the soybean organism produced a reaction unlike that on soybean. No pustules were formed and sections through a spot 17 days after inoculation showed neither hypertrophy nor hyperplasia. Bacterial cavities and masses of bacteria were present. In only one case out of many was there observed even a slight elevation of the epidermis. Furthermore, if the infection made any progress after the appearance of the first small red spots or pale green ones with red centers, the spots became water-soaked and could not be distinguished from those caused by *Bact. phaseoli* EFS. In the best infections there was also yellowing in the later stages and a distortion of the very young leaves. In general, *Bact. phaseoli sojense* infected Lima bean leaves much more slowly than did the organism described from Phaseolus (Pl. 4, C, D, and H) except when the former's virulence for Lima was apparently increased by previous passage through Lima or bush string bean. The tiny spots sometimes did not appear for 2 weeks or more and then were very scattering and not infrequently failed to increase in size (Pl. 4, C). The plants were inoculated in the greenhouse by spraying and were kept moist in inoculation cages 48 to 72 hours, as a hot moist atmosphere is very favorable to infection of Phaseolus with both *Bact. phaseoli* EFS. and *Bact. phaseoli sojense*.

The first good infections on Lima beans were obtained on the green pods by spraying them July 13, 1917, with a water suspension of a 2-day-old potato culture of the soybean organism and keeping them wrapped in oiled paper 7 days. One side only had been sprayed, and when the paper was removed this side was covered with tiny water-soaked spots while the other was perfectly free. Three days later these water-soaked spots were very marked and there were still none on the unsprayed side. Bacterial ooze was also present. Later, the spots turned red but they enlarged little, if any, after the first 10 days (Pl. 4, E, F). The parasite was reisolated, and one of the resulting colonies produced excellent infections on both soybean leaves and pods, and on Lima bean pods, which were treated as described above. The Lima bean leaves were not inoculated.

INOCULATIONS ON DWARF BUSH STRING BEANS

Dwarf bush string bean was much more susceptible than Lima (with the exception of the latter's pods) to *Bact. phaseoli sojense* previous to its passage through a species of Phaseolus. The young leaves of this variety inoculated in the greenhouse September 30, 1919, were thickly covered with water-soaked spots on the ninth day after inoculation (no records made earlier). Two days later the center of the spot was becoming discolored but there was never much, if any, enlargement of it thereafter, nor was there any yellowing (Pl. 4, A). The spots were full of bacteria and the parasite was reisolated from them. These inoculations were made on plants 2 weeks old in an inoculating cage in which they were kept moist for 72 hours. There was very slight infection on King of the Garden Lima plants 3 weeks old inoculated at the same time,



Bacterial Pustule of Soybean

Plate 3

Bacterium phaseoli sojense. (Photographs by JAMES F. BREWER)

A to D.—Sections of pustules 3 weeks old produced by inoculation; bacteria throughout. A.—Hypertrophy and hyperplasia; bacterial lesion (X), ×160. B.—Detail from A. ×900. C.—Pustule on lower leaf surface; a few bacteria penetrating normal tissue also. ×200. D.—Elongation of epidermal cells in pustules. ×260.

E.—*Bacterium phaseoli sojense* from 24-hour-old beef agar slant. Casares-Gil's flagella stain. ×2000.

in the same manner with the same culture, which, furthermore, produced good typical infections on soybean.

When previously passed through Phaseolus the soybean organism produced infections on bush string bean leaves and pods (Pl. 1, H, I; Pl. 4, B) in no way distinguishable from those caused by *Bact. phaseoli* EFS. (Pl. 1, J, to L).

INOCULATIONS WITH BACTERIUM PHASEOLI SOJENSE AFTER PASSAGE THROUGH PHASEOLUS

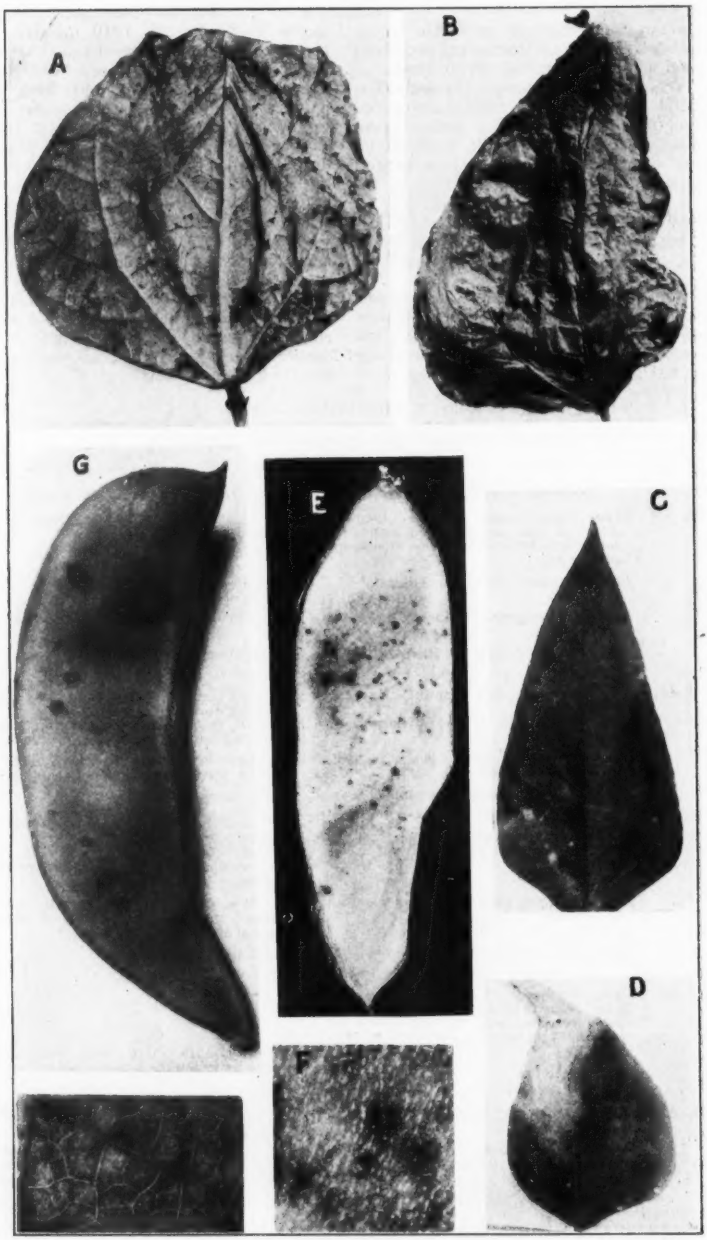
As it had occurred to the writer that the pathogenicity of *Bact. phaseoli sojense* for Phaseolus might be increased by its passage through members of that genus, parallel inoculations were made September 30, 1919, on King of the Garden Lima (3 weeks old), dwarf bush string bean (2 weeks old) and Hahto soybean (3 weeks old) with two cultures of *Bact. phaseoli sojense*, one (colony 3) plated May 26, 1919, from soybean, the other (colony 1) plated July 19, 1919, from a Lima leaf, but both descended originally from the same colony plated from the Texas material received in 1916.[5] The inoculations were made by spraying in inoculating cages in which the plants were kept moist for 72 hours. In addition to the above, six King of the Garden Limas (3 weeks old) were sprayed, three with each organism, and covered with paraffined bags for 72 hours. The results were as follows:

BACT. PHASEOLI SOJENSE

Inoculation experiment showing the effect of previous passage through Phaseolus

	COLONY 3 (FROM SOYBEAN)	COLONY 1 (FROM LIMA BEAN)
9th day	*Lima bean* (cage): Infection doubtful. Tiny red spots and distorted leaflets but no bacteria found in either on microscopic examination.	*Lima bean* (cage): Good infection, water-soaked spots.
	Lima bean (bags): Infection doubtful.	*Lima bean* (bags): Water-soaked spots. Bacteria in abundance.
	Dwarf bush string bean: Peppered with water-soaked spots.	*Dwarf bush string bean:* Water-soaked spots.
	Soybean: Typical infection, pustule stage.	*Soybean:* Infection doubtful.
11th day	*Lima bean* (cage): Still doubtful. Found no bacteria. *Lima bean* (bags): A few tiny suspicious-looking spots.	*Lima bean* (cage): Excellent infection. Spots becoming discolored. *Lima bean* (bags): Excellent infection. Some of infected leaves are wilting and shrivelling.
	Dwarf bush string bean: Centers of spots becoming discolored.	*Dwarf bush string bean:* Excellent infection on a few leaves—discoloration beginning.
	Soybean: Center of pustules becoming brown.	*Soybean:* Infection doubtful.
15th day	*Lima bean* (cage): No typical spots. *Lima bean* (bags): Infection doubtful.	*Lima bean* (cage): Spots increasing in size. *Lima bean* (bags): Spots increasing in size and surrounding tissue becoming yellow. Some shrivelling.
	Dwarf bush string bean: Spots have increased little, if any, in size.	*Dwarf bush string bean:* Conspicuous spots, increased in size; distortion of leaf due to infection of veins, shrivelling.
	Soybean: Typical infection.	*Soybean:* Infection doubtful.
18th day	*Lima bean* (cage): No typical spots but found bacteria in the distorted leaflets and pale green spots with tiny red centers and *isolated the parasite from both.*	
		Dwarf bush string bean: Some wilt also. *Isolated parasite* from petiole of wilted leaf.
27th day	*Lima bean* (cage and bags): No change.	*Lima bean* (cage and bags): Excellent infection in all. Shrivelled leaves.
	Dwarf bush string bean: No enlargement of spots which are still small and red. No yellowing. Small red spots full of bacteria. *Parasite isolated.* Photographed leaf. (Pl. 4, A).	*Dwarf bush string bean:* Some leaves shrivelled. Some have dropped off. Multitude of bacteria. *Parasite isolated.* Photographed leaf. (Pl. 4, B).
	Soybean: Typical infection	*Soybean:* No signs of infection

[5] These two colonies had passed through the following series of inoculations:
From Texas soybean: Colony e, Sept. 23, 1916>soybean>Lima pod>soybean>{soybean>colony 3. / soybean>Lima>colony 1.



Bacterial Pustule of Soybean

Plate 4

(For explanatory legend see p. 237)

BACT. PHASEOLI SOJENSE—Continued

Inoculation experiment showing the effect of previous passage through Phaseolus—Continued

	COLONY 3 (FROM SOYBEAN)	COLONY 1 (FROM LIMA BEAN)
5th week	*Lima bean* (cage and bags): No noticeable increase in infection. The two worst infected leaves are much distorted but are neither yellowed nor shrivelled.	*Lima bean* (cage and bags): Striking infection. A number of leaves have dropped off, wholly browned and shrivelled or with only a small amount of green tissue remaining, as sometimes occurs in inoculations with *Bact. phaseoli* EFS. (Pl. 1, L). Others have large brown shrivelled areas bordered with yellow, involving one-half to three-fourths of the leaflet.
	Dwarf bush string bean: Spots somewhat larger than at time of previous observation but there has been no very noticeable spread of the infection.	*Dwarf bush string bean:* Conspicuous infection. Typical brown and shrivelled spots, bordered with yellow.
	Soybean: Spots have increased considerably in size.	*Soybean:* No signs of infection.
8th week	*Lima bean:* no secondary infections.	*Lima bean:* Conspicuous infections in every respect like those caused by *Bact. phaseoli* EFS.
	Dwarf bush string bean: No increase of symptoms. Infection is much more restricted than on plants inoculated with colony 1.	*Dwarf bush string bean:* Not distinguishable from infections caused by *Bact. phaseoli* EFS.
	Soybean: Spots enlarging somewhat. There has been good infection.	*Soybean:* No infection.

A repetition of the experiment with the same colonies corroborated the results set forth above.

A third set of inoculations was made with another pair of colonies[6] but the temperature of the hothouse (about 62° F.) was too low to favor infection.

In the parallel sets of inoculations here described, passage through Phaseolus seemed not only to increase the pathogenicity to Phaseolus but to destroy it for soybean. However, in two previous experiments excellent infections had been obtained on soybean by colony 1, August 6, 1917, through Lima (Pl. 1, E, F; Pl. 2, B, D); and in two later experiments with colonies isolated from bush string bean some scattering but typical infections were obtained on soybean (Pl. 1, G). With one of these colonies[7] excellent infections were produced on bush string bean, dwarf wax bean and dwarf bountiful string bean. No infection was obtained on dwarf wax or Early Refugee wax bean with the other colony,[8] but wilt was caused on dwarf bountiful string bean. (Though primarily causing leafspot, both *Bact. phaseoli sojense* and *Bact. phaseoli* EFS. may produce wilt if the organism invades the vascular system.)

In conclusion, however, it may be said that *in no case has the writer found the same colony of Bact. phaseoli sojense equally infectious to soybean and Lima or bush string bean.*

[6] From Texas soybean: Colony e, Sept. 23, 1916>soybean>Lima pod>soybean>soybean> { soybean>colony 5 / bush string bean>colony 12.

[7] From Texas soybean: Colony e, Sept. 23, 1916,>soybean>Lima pod>soybean>soybean>Lima>bush string bean>colony 6, Oct. 20, 1919.

[8] From Texas soybean: Colony e, Sept. 23, 1916>soybean>Lima>bean pod>soybean>soybean>bush string bean>colony 11, Dec. 5, 1919.

EXPLANATORY LEGEND FOR PLATE 4

Inoculations on Phaseolus with *Bacterium phaseoli sojense* (through soybean), *Bacterium phaseoli sojense* (through Lima bean), and *Bacterium phaseoli* EFS. for comparison. (Photographs by JAMES F. BREWER.)

A and B.—Comparative effect on bush string bean of *Bacterium phaseoli sojense* four weeks after inoculation with (A) organism through soybean; small spots; (B) organism through Lima bean: leaf shriveled ×1⅓.

C and D.—Comparative virulence to Lima of (C) *Bacterium phaseoli sojense* through soybean and (D) *Bacterium phaseoli* EFS. 18-day-old infections. ×1. C, tiny spots, no water soaking. D, excellent typical infection.

E to G.—Comparative effect on Lima pods of (E–F) *Bacterium phaseoli sojense* through soybean and (G) *Bacterium phaseoli* EFS. E, 3-weeks-old infection. ×1. F, detail from E. ×5 ca. G, 7-day-old infection. ×1.

H.—*Bacterium phaseoli* EFS. 11-day-old infection on Lima bean leaf, water soaking. ×5.

It is to be noted that the infection on all the varieties of Phaseolus inoculated with *Bact. phaseoli sojense* resembled that produced by *Bact. phaseoli* EFS. and in the best cases was not to be distinguished from it. There was even reddening of the veins in infected dwarf bountiful string bean, but *in no case were pustules produced.* Wilt was occasionally observed in infections on Phaseolus. Microscopic examination of the petiole of a wilted bush string bean leaf showed that the bacteria were present in the vessels but not confined to them.

COMPARATIVE INOCULATIONS ON BEANS AND SOYBEANS WITH BACTERIUM PHASEOLI EFS. AND BACTERIUM PHASEOLI SOJENSE (THROUGH SOYBEAN)

Not only is *Bact. phaseoli* EFS. much more infectious to Phaseolus than the soybean organism, but thus far, with the exception of a single experiment, it has been entirely nonpathogenic to soybean seedlings or older plants, when inoculations have been made by any of the methods so successful in producing infection on Phaseolus with *Bact. phaseoli* EFS. or soybean by inoculation with *Bact. phaseoli sojense.* Infection was obtained when a modification of these methods was employed, that is, when the leaves were rubbed gently between thumb and forefinger after spraying. In addition to spraying and pricking inoculations in inoculation cages, an attempt was made to produce the disease by so placing healthy young soybeans among very badly diseased Lima beans that their leaves would come in contact with the bacterial ooze from the leaves of the latter, and by watering the plants in such a way that the bacteria would be washed down upon the soybeans. With the single exception noted above, all of these inoculations failed. There were 24 of these experiments, made in every month except May and November.

The history of the exceptional inoculation experiment is as follows: Eight pots of Mammoth Yellow soybean seedlings grown from seeds treated with concentrated H_2SO_4 and planted September 15, 1920, were sprayed at 4 p. m. October 4 with a 2-day-old culture of what proved later to be a very virulent colony of *Bact. phaseoli* EFS. (colony 6, August 24, 1920, isolated from string bean leaves). The seedlings ranged in size from those just appearing above the ground to plants 8 or 9 inches high. Some were pricked as well as sprayed. The plants were sprayed again the following day at 10 a. m. and 4 p. m. and were kept in a moist atmosphere for 45 hours. On October 15, 11 days after inoculation, there were a few scattering brown spots, some of them marginal. These were not associated with the pricks. There were no pustules and the spots did not resemble infection by *Bact. phaseoli sojense* nor did microscopic examination reveal the presence of bacteria. Plates were poured, however, and *Bact. phaseoli* EFS. isolated, but although the plates were inoculated very heavily only seven colonies appeared. Two of these showed the radiating lines, and two the internal concentric striae both illustrated in Plate 7, B and J. In other spots examined later a few scattering bacteria were found. The browning may have been partly due to asphyxiation; yet in both this experiment and in some described later *Bact. phaseoli* EFS. was plated from the browned areas.

This very slight evidence of the pathogenicity of *Bact. phaseoli* EFS. for soybean led to some experiments which resulted in very good infections on soybean, but the conditions were quite abnormal. The inoculation experiment described below gives a fair idea of the typical differences usually found between *Bact. phaseoli sojense* and *Bact. phaseoli* EFS. in their pathogenicity to Lima bean and soybean:

Three rapidly growing Lima bean plants (Burpee's Fordhook Lima) just beginning to put forth tiny flower buds and two Hahto soybean seedlings 8 to 12 inches high were inoculated June 5, 1919, by spraying with *Bact. phaseoli* EFS. In other experiments many more soybeans were inoculated, but with the same results. For comparison eight large Hahto soybeans and two seedlings of the same variety about 8 inches high, also one Lima bean plant with several pods and blossoms, were inoculated with the soybean organism (from soybean). In other experiments more Lima beans were used, but with the same results. Two colonies of each organism were used, and both sets of plants were put into inoculation cages and kept in a very hot, moist atmosphere for two days. The results were as follows:

	BACTERIUM PHASEOLI SOJENSE (THROUGH SOYBEAN)	BACTERIUM PHASEOLI EFS.
8th day.	*Soybean* seedlings: pale green spots, pustules. Large plants: no signs of infection. *Lima:* No signs of infection.	*Soybean* seedlings: No signs of infection. *Lima:* Excellent infections on all plants. On some of the younger leaves there is scarcely any sound tissue and the leaves are distorted. The leaves have a pale green mottled appearance on the surface and are a mass of water-soaked spots underneath.
11th day.	*Soybean:* No noticeable change. Both colonies infectious. *Lima:* No signs of infection.	*Soybean* seedlings: No signs of infection. *Lima:* Worst infected leaves are rapidly shrivelling. Spots brown and surrounding tissue is turning yellow.
13th day.	*Soybean* seedlings: Spots larger and considerably more conspicuous. Larger plants: No signs of infection. *Lima:* No signs of infection.	*Soybean* seedlings: No signs of infection. *Lima:* Bacterial ooze on the infected leaves. The flowering has been much retarded, buds still very tiny while checks are in full flower. Brown spots coming on stems of all plants.
16th day.	*Soybean:* No infection on older plants. *Lima:* No signs of infection.	*Soybean* seedlings: No infection. *Lima:* Distortion of many of the younger diseased leaves where enough contiguous sound tissue was left to continue to grow normally while the diseased area could not. Many yellowed leaves. Many leaves dropping off.
18th day.	*Soybean:* No infection on older plants. *Lima:* No signs of infection.	*Soybean:* No signs of infection. *Lima:* In some of the leaves practically all the chlorophyll is gone—only isolated patches of green. The rest of the leaf is yellow, not brown or shrivelled or dry.
19th day.	*Soybean:* No infection on older plants. *Lima:* No signs of infection.	*Soybean:* No infection. *Lima:* Excellent stem infections—external brown spots filled with bacteria. Flower buds not opening. Many buds falling off.
5th week.	*Soybean:* Infection on older plants also. *Lima:* A few infections. These are like typical *Bact. phaseoli* EFS. infections. Multitudes of bacteria.	*Soybean* seedlings: No infection.
6th week.	*Lima:* Many water-soaked spots on some of the leaves. This water-soaking is less noticeable to the naked eye than that in young spots on Lima produced by *Bact. phaseoli* EFS. Slight elevation of the epidermis but no real pustules.	*Soybean* seedlings: No infection.
7th week.	Soybean photographed (Pl. 2, C).	

Later comparative inoculations on Lima bean in which younger plants were inoculated with the two organisms produced similar results (Pl. 4, C, D). Infection with the soybean organism (through soybean) took place very slowly. The water-soaking was usually absent or much less conspicuous than in infections with *Bact. phaseoli* EFS. and sometimes the only signs of infection for weeks were tiny pale green spots with or without red centers. Usually, however, a few spots resembling typical *Bact. phaseoli* EFS. infections appeared weeks after inoculation.

In both these sets of comparative inoculations with *Bact. phaseoli sojense* (through soybean) and *Bact. phaseoli* EFS. the difference in virulence for Lima bean was very striking throughout the experiments.

INFECTION PRODUCED ON SOYBEAN SEEDLINGS BY BACTERIUM PHASEOLI EFS.

The first very successful infections on soybeans with *Bact. phaseoli* EFS. were obtained on the cotyledons of Ito San soybeans germinated and inoculated in a sterile Petri dish damp

chamber. The seeds were treated with formaldehyde by the presoak method [9] described by Braun (*1*) and put into the damp chamber November 24, 1920. Two days later, having germinated, they were inoculated by spraying with a water suspension of a 2-day old culture of *Bact. phaseoli* EFS. The following day they were sprayed again. No leaves were yet visible. On the third day after inoculation some of the leaves were beginning to appear and the seedlings were transferred to large moist chambers.

On November 30 there were no signs of infection and five of the seedlings (Nos. 16 to 20) were transferred to Sachs' nutrient solution and placed under a bell jar in a south window in the laboratory. On December 2 they were reinoculated by spraying. The leaves were just beginning to be visible between the cotyledons in some cases; in others they were beginning to push out.

Eight days after the first spraying in the Petri dish the cotyledons of many of the seedlings remaining in the damp chamber had water-soaked spots and bacterial ooze therefrom (Pl. 7, N, O). In some cases the seed coat was still firmly attached and the spots were visible through it. Seven of these seedlings with infected cotyledons (Nos. 45–51) were transferred to Sachs' solution and placed under a bell jar in a north window in the laboratory. They were sprayed heavily with sterile H_2O in an attempt to spread the infection by scattering the bacteria oozing from the spots.

On December 9 seedlings Nos. 16 to 20, reinoculated December 2 (two days after their transfer to Sachs' solution), showed a pronounced browning and curling of the leaf tips or margins (like Pl. 7, P). Five days later *Bact. phaseoli* EFS. was isolated from the browned curled margin of a leaf of No. 20 in which microscopic examination showed the bacteria present in abundance.

Also on December 9 seedlings Nos. 45 to 51, transferred to Sachs' solution on December 4 but not reinoculated, were all very much stunted. The main shoot was shriveling in Nos. 48 to 51 and there were irregular brown spots on the leaves of the other three (Nos. 45 to 47). *Bact. phaseoli* EFS. was reisolated December 11 from dark brown spots on the cotyledons of plant No. 46. On December 31, all the plants transferred to Sachs' solution were dead, doubtless partly because of the abnormal conditions under which they had been grown.

Similar results were obtained on Ito San soybean seed treated with sulphuric acid [10] and inoculated by spraying in a Petri dish damp chamber immediately after germination therein.

In a third experiment presoaked soybean seeds of the Ito San variety, treated with formaldehyde, were germinated in a damp chamber and transferred as soon as the seedlings had made sufficient growth to Sachs' solution and inoculated with *Bact. phaseoli* EFS. 8 days after the sowing of the seed in the damp chamber. In some cases the leaves had not begun to push out, but the cotyledons were spreading and the culture was sprayed between them upon the folded leaves at 2.15 p. m. They were placed under a low bell jar in a south window of the laboratory; at 4.15 they were sprayed again and twice the next day, at 10.20 a. m. and 4.30 p. m. The moisture held well for 48 hours. The low bell jar was then replaced by a taller one and this was removed 2 days later. A week after the inoculation there was a curling and yellowing or browning of the margin of many of the first leaves (like Pl. 7, P), and *Bact. phaseoli* EFS. was isolated from two of them. No infection of cotyledons was observed.

Ito San soybean seedlings grown like the preceding but taken to the greenhouse, after their transfer to Sachs' solution, and there inoculated eight days after the seed was sown in the damp chamber, became similarly infected. One of these photographed eight days after inoculation, is shown in Plate 7, P.

These experiments were repeated several times and always resulted in infection. *Bact. phaseoli* EFS. was repeatedly isolated both from the cotyledons and the brown curled leaf margins. *No pustules were ever produced.*

Another attempt was made on December 10, 1920, to produce infection with *Bacterium phaseoli* EFS. on young seedlings of Ito San and Mammoth Yellow soybeans growing in pots in the greenhouse, but the only positive results were obtained on 11 Ito San seedlings, the leaves of which had been rubbed

[9] H_2O for 10 minutes in bags of double thickness surgeon's gauze; moist chamber (still in bags), 6 hours; 1:300 formaldehyde for 15 minutes; moist chamber (wet with 1:300 formaldehyde), 45 minutes; spread out to dry overnight.

[10] H_2SO_4 (concentrated) for 1 minute; H_2SO_4 poured off and sterile water added; sterile water added and poured off several times.

gently between the thumb and forefinger after spraying with the bacterial suspension. There were 23 Ito San and 61 Mammoth Yellow soybean plants used in the experiment. The Ito San seed had been treated with formaldehyde and germinated in a damp chamber, then planted in pots. The plants had developed the first pair of leaves when inoculated. The Mammoth Yellow seed was untreated and germinated in the soil. The cotyledons were just beginning to spread, showing a leaf shoot at the time of inoculation.

The plants were kept moist in inoculating cages, bell jars, or paraffined bags for 28 hours after spraying with *Bact. phaseoli* EFS. On the rubbed leaves of the 11 infected Ito San seedlings were narrow brown streaks and pale green areas, from the former of which the parasite was reisolated. Twelve other Ito San seedlings and 32 Mammoth Yellow seedlings were in the same inoculating cage, but no brown streaks appeared nor did they ever show any signs of infection. In this case it seems reasonable to conclude that the brown streaks were due to infection rather than to asphyxiation.

Another method which proved successful was that of dipping the seed of Ito San soybean treated by the H_2SO_4 method, in a water suspension of the organism and germinating it in a damp chamber. The parasite was reisolated from a water-soaked cotyledon on one of these soybeans. For this type of inoculation *Bact. phaseoli* EFS. isolated from soybean was used.

INOCULATION OF SOYBEAN AND LIMA BEAN WITH BACTERIUM PHASEOLI EFS. AFTER PASSAGE THROUGH SOYBEAN AND VELVET BEAN

On the assumption that passage through soybean might increase the pathogenicity of *Bact. phaseoli* EFS. to soybean, spray inoculations were made upon seedlings of this plant and of Lima bean in inoculation cages. But passage of *Bact. phaseoli* EFS. through soybean (*Glycine hispida*) or Georgia velvet bean (Stizolobium) did not increase its virulence for soybean, nor, on the other hand, did passage through soybean decrease its pathogenicity to Lima bean, as evidenced by the fact that the worst cases of infection which the writer has ever seen were produced on King of the Garden and Fordhook Bush Lima sprayed in the hothouse in February with *Bact. phaseoli* EFS. isolated from inoculated soybean. The high temperature maintained in this house, where the thermometer rarely fell below 75° F. during the night, and for days reached 103 to 110° in the middle of the day, was in a very large measure responsible for this. The difference in the severity of the infection on the plants in this house and on plants of the same variety and age, inoculated at the same time with the same culture, but kept in a house with a temperature ranging from 64° to 72° F. the greater part of the time, was striking in the extreme. In the hotter house infection was much more abundant and progressed farther and much more rapidly. One of the worst infected plants collapsed entirely. The whole top was dead and the stem was withered and full of bacteria. The infection of the stem extended all the way down to the ground from the first pair of leaves, a distance of 6 inches. There were drops of yellow ooze from the stem when squeezed. These plants were inoculated when they had only the first pair of leaves.

CULTURAL CHARACTERS

In all cultural work on the soybean organism parallel tests were made with *Bact. phaseoli* EFS., but inasmuch as with a few exceptions no greater differences were found than might exist between two colonies of the same organism, the reader is referred to Smith's published descriptions of the cultural characters of *Bact. phaseoli* EFS. (*15, 16, 17, 20, p. 285–287*) and space will be given here only to the differences found and to some additional facts not hitherto recorded.

The best medium found for the soybean organism is fresh steamed potato cylinders. *Bact. phaseoli sojense*, like *Bact. phaseoli* EFS., makes a very copious growth on this medium, consuming practically all the starch, and so filling the 2 cc. or more of water around the cylinders with slime that it becomes a solid yellow mass and does not flow when the tubes are turned upside down. The writer has never failed to obtain this characteristic growth if the potato cylinders were fresh, but even a slight drying out of the upper part of the cylinder may alter the character of the growth.

Much of the cultural work was done before P_H determinations were made in this laboratory, and "approximately" after the P_H reading means that the latter was worked out from the Fuller's scale reading according to the formula of Quirk and Fawcett (*12, p. 20*).

The descriptions which follow relate equally to *Bact. phaseoli sojense* and *Bact. phaseoli* EFS. unless otherwise noted:

BEEF AGAR SLANTS.—(+15 Fuller's scale, P_H 6.8, actual determinations). Moderate pale yellow, flat, filiform, smooth, glistening, translucent growth in 24 hours. No distinctive odor. May become slightly viscid. Growth increases somewhat but is never copious.

BEEF AGAR STABS.—(+15 Fuller's scale, approximately P_H 6.8).

(All the beef agars used were made with beef infusion +1 per cent Difco peptone.) Best growth at top. Surface growth good, nearly covering surface of agar, nailhead slightly convex. Line of puncture filiform. Never very much growth at bottom of the stab.

GILTNER'S CONGO RED [11] AGAR.—Very thin, colorless, blisterlike growth in 24 hours. In 3 to 4 days there is a thick, smooth, wet-shining growth along the line of the stroke which has absorbed the red but has a slightly yellowish tinge. In six days the surface of the slant is nearly covered by a copious growth. Agar becomes purplish in 2 to 6 weeks, a decided contrast to the check. An excellent medium for this organism.

SYNTHETIC AGAR.—(Like the preceding without the Congo red). Excellent growth as in the above. Very little yellow pigment. Pigment best seen by oblique light.

LITMUS DEXTROSE AGAR.[12]—Good growth along the path of the streak in 2 days. No acid or alkali produced in 4 days and no reduction of the litmus. Considerable alkali produced in 3 weeks.

LITMUS LACTOSE AGAR.[13]—Thin growth covering the surface of the slant in 2 days. No change in the color of the agar in 3 to 4 days. Slight bluing in 5 days. Decided bluing in 8 to 10 days. Growth greenish yellow and not copious (10 days). In 3 to 4 weeks the blue of the agar had a decidedly greenish tinge. There was never any reduction of the litmus. (Under observation 4½ months.)

LÖHNIS SOIL EXTRACT AGAR.[14]—Barely visible growth in 24 hours. Still a very scanty growth in 5 weeks.

POTATO AGAR [15] PLUS 10 PER CENT DEXTROSE.—Good growth along the path of the streak in 24 hours. Excellent, smooth, yellow, wet-shining growth in 2 days. All the potato starch consumed by the sixteenth day.

WHEY AGAR.[16]—Good to copious, wet-shining, yellow growth, sometimes piled up along the streak in 3 days. Surface of slant nearly covered in 6 days. In one set of 9-day-old cultures *Bact. phaseoli sojense* was amber yellow and *Bact. phaseoli* EFS. primuline yellow (*13*).

LOEFFLER'S BLOOD SERUM.—Excellent growth. Liquefaction begins in 6 to 9 days, good but not complete liquefaction in 2½ months.

NUTRIENT GELATIN.—(+10 to +12 Fuller's scale, P_H 7.2 to 7, actual determinations.) Beef infusion bouillon, 1 per cent peptone, 10 per cent gelatin. Gelatin liquefied promptly, somewhat more rapidly by *Bact. phaseoli sojense* than by *Bact. phaseoli* EFS. Liquefaction begins in 2 to 3 days and is usually stratiform.

BEEF BOUILLON.—(+12 to +15 Fuller's scale, P_H 7.2 to 6.9, actual determinations.) Thin to moderate clouding in 24 hours, with rolling clouds on shaking, partial rim, no pellicle or sediment. Flocculence and heavy yellowish rim stringing down into the liquid in 4 or 5 days. In 3 to 4 weeks there is a partial or complete pellicle which may be thin or heavy, stringing down into the liquid and shaking down easily; and the culture medium is clearing. Viscid yellow precipitate in cultures two or more weeks old.

FERMENTATION TUBES.—Tested the organism in 1 per cent solutions of saccharose, maltose, mannit, glycerin, dextrose and lactose in 1 per cent Difco peptone water. No gas in any. Good clouding in open end in all. Faint clouding in closed end in lactose in 7 days. Titration at the end of 18 days showed an increase of acid in the cultures—most in maltose, saccharose, and dextrose.

[11] 1,000 cc. distilled water; 10.0 gm. saccharose; 1.0 gm. dipotassium phosphate; 0.2 gm. magnesium sulphate; 15.0 gm. agar flour; 0.1 gm. Congo red. Water and all salts steamed for 30 minutes. Congo red then added. Filtered through cotton and tubed. Autoclaved 15 minutes at 110° C.

[12] 1 per cent beef infusion agar+1 per cent Difco peptone+litmus+1 per cent dextrose.

[13] 1 per cent beef infusion agar+1 per cent Difco peptone+litmus+1 per cent lactose.

[14] 150 gm. soil; 1,000 cc. distilled water; trace calcium oxide; 0.5 gm. dipotassium phosphate; 10.0 gm. mannite; 15.0 gm. agar; excess calcium carbonate.

[15] 750 cc. potato juice; 10 per cent dextrose; 2 per cent agar. Steamed 45 minutes. Filtered through cotton. Autoclaved 15 minutes at 110° C.

[16] Fresh milk boiled gently for 5 minutes, 20 per cent HCl added drop by drop till all casein was precipitated. (An excess of acid is to be avoided.) Coagulum removed by straining through cheesecloth and the whey made exactly neutral to litmus (neutral litmus) with 20 per cent solution of sodium hydroxide. To each liter of whey was added: 300 cc. distilled water; 3 gm. gelatin; 15 gm. cane sugar; 15 gm. peptone; 15 gm. agar. Steamed 30 minutes.

USCHINSKY'S SOLUTION.—Faint to moderate clouding. Medium in old cultures (3½ to 4 months) is the color of beef broth. Cultures of *Bact. phaseoli sojense* sometimes become reddish brown.

LITMUS MILK.—Reduction of litmus beginning in 4 to 16 days, complete in 5 to 19 days (occasionally not complete in 3 weeks). Otherwise as recorded in descriptions cited.

MILK PLUS METHYLENE BLUE.—All color disappears in 1 to 2 days. Decided yellowish cast to whey and coagulum in 3 weeks.

SOYKA'S RICE MILK.—Bright yellow growth.

TOLERATION OF ACID AND ALKALI [17]

This work was done in conjunction with Quirk and Fawcett in their work on "Hydrogen-ion concentration vs. titratable acidity in culture mediums" (*12, p. 21, 50, 54*).

Tests of acid and alkali toleration were made in 1 per cent peptone beef broth (hot infusion A (*12, p. 21*)), to which NaOH or HCl was added. Growth occurred in 24 hours in 0 to +17 Fuller's scale (8.1 to 6.5 P_H)[17] and in 72 hours *Bact. phaseoli* EFS. had grown in −3 to +28 Fuller's scale (8.4 to 5.7 P_H)[17], and *Bact. phaseoli sojense* in 0 to +28 (8.1 to 5.7 P_H).[17] In 2 weeks both had clouded the broth which titrated, at the time of inoculation, −6 Fuller's scale (8.7 P_H).[17] A titration of check tubes, however, showed that the acidity of this medium had increased considerably upon standing. At the end of a week the check titrated −3 Fuller's scale (8.3 P_H)[17] and at the end of 2 weeks, at which time growth was noted, the check titrated −1 Fuller's scale (8.2 P_H).[17] There was still no clouding of +32 Fuller's scale (5.238 P_H).[17] A titration of a check tube showed that the acidity had changed very little on standing 2 weeks. The check titrated +33 Fuller's scale (5.2, 5.16 P_H).[17]

TOLERATION OF SODIUM CHLORIDE

Slight growth in beef bouillon neutral to phenolphthalein plus 4 per cent NaCl. Transfers made from the cultures to potato on the thirtieth day produced no growth.

PRODUCTION OF AMMONIA AND INDOL

Ammonia was produced in beef bouillon (+12 Fuller's scale, P_H 5.8, actual determinations) tested on the twenty-second day with Nessler's solution. No indol was found in Uschinsky plus peptone. The sodium nitrite-sulphuric acid test was made on the twenty-first day, but there was no pink reaction even upon heating.

VARIETAL DIFFERENCES BETWEEN BACT. PHASEOLI SOJENSE AND BACT. PHASEOLI EFS. IN PLATE CULTURES

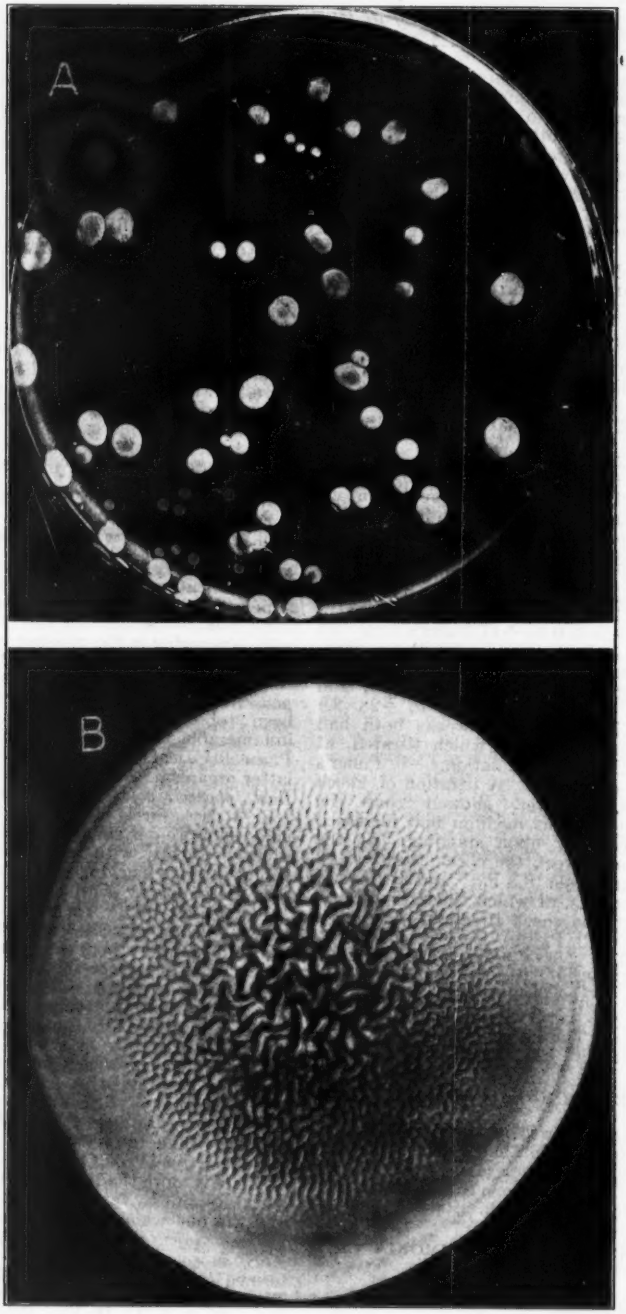
In the preceding tests *Bact. phaseoli sojense* was not distinguishable culturally from *Bact. phaseoli* EFS. There have, however, been some interesting differences in the behavior of the colonies on beef-agar plates.

COLONIES OF BACT. PHASEOLI SOJENSE ON 1 PER CENT BEEF AGAR (MADE WITH BEEF INFUSION + 1 PER CENT PEPTONE AND 2 PER CENT AGAR) PLATES AT ROOM TEMPERATURE (PL. 5 AND 6)

Brief mention was made in a previous paper (*9*) of certain internal markings very commonly, though not universally, appearing in the colonies of *Bact. phaseoli sojense* which serve to distinguish it in a measure from *Bact. phaseoli* EFS. inasmuch as they have been observed but twice in the innumerable isolations made from the Phaseolus leafspot caused by the latter organism. When the colonies of *Bact. phaseoli sojense* first appear (in 2 or 3 days, rarely 4 or 5) they are pale yellow, round, smooth, wet-shining, slightly convex, with entire margins and no internal markings. At this time they are in no way distinguishable from *Bact. phaseoli* EFS. They also become a deeper yellow with age like the former, but in 5 to 7 days there very commonly appear in the surface colonies on the more thinly sown plates the aforesaid markings which are visible for 1 to 2 weeks (rarely 3 weeks). They are wholly internal, the surface of the colony remaining smooth. They are usually seen only by oblique transmitted light and a hand lens (Zeiss ×6) but they are sometimes so distinct that they are plainly visible by reflected light and the naked eye (Pl. 5, A).

There is more than one type of these markings, but by far the most common and the one which distinguishes *Bact. phaseoli sojense* from *Bact. phaseoli* EFS. is one which the author has termed *internal convolutions* (Pl. 5, B). There are various modifications of this type (Pl. 6, A, C, D, F). When they

[17] Potentiometer determinations.



Bacterial Pustule of Soybean Plate 5

Bacterium phaseoli sojense. Colonies with internal convolutions. (Photographs by JAMES F. BREWER)

A.—Beef agar plate from small red spots on bush string bean, 3 weeks after inoculation (Pl. 4, A). Convolutions visible to naked eye. 8 days old. ×1.

B.—Plated from pale green spots with red centers on Lima bean leaf 12 days after inoculation. Colony 13 days old. ×6 ca.

were first observed (Pl. 6, A) they were called *reticulations*, but this term does not as well describe the appearance in most of the later isolations as does "convolutions."

Another rather common type of markings is "radiating lines." These are sometimes very distinct (Pl. 6, B). sometimes much less striking (Pl. 6, G).

A third and much rarer type for *Bact. phaseoli sojense* is that of "concentric striæ" (Pl. 6, E).

Very often more than one of these types is found on the same plate and, on the other hand, the author has never seen a plate in which there were not some surface colonies with no markings at all. The markings have never been observed on very thickly sown plates.

Thus far no explanation of the appearance or nonappearance of these markings has been found. The following observations have been made:

AGE AND TITRATION OF THE BEEF AGARS USED.—The variations in the age and reaction of the agar apparently have no effect on the type of colony produced.

Convoluted colonies.—On agar 2 to 16 days old at the time of inoculation and titrating +10 to +15 Fuller's scale (P_H7.2 to P_H6.7 approximately). These figures refer to the titration immediately after the final sterilization of the agar. +10 to +15 Fuller's scale beef agar may become 2° (Fuller's scale) more acid upon standing 2 weeks.

Colonies with radiating lines.—On agar 2 to 16 days old at the time of inoculation and titrating +11 to +15 Fuller's scale (P_H7.1 to P_H6.7, approximately).

Concentrically striated colonies.—On agar 4 to 9 days old at the time of inoculation, titrating +14 to +15 Fuller's scale (P_H6.8 to P_H6.7 approximately).

Plates containing no colonies with markings of any kind.—On agar 1 day to 3 months old at the time of inoculation, titrating +14 to +15 Fuller's scale (P_H6.8 to P_H6.7, approximately).

The fact that both marked and unmarked colonies, and colonies with various types of markings, appeared on the same plates would indicate at the outset that in these experiments the variations in the agar used had little, if any, influence. The marked colonies were in all portions of the plates (Pl. 5, A), hence a varying depth of agar because the plates were not entirely flat on the bottom could not be the cause of colony variation.

LENGTH OF TIME IN THE PLANT (THAT IS, TIME OF INOCULATION TO TIME OF ISOLATION).—All types were found in both young and old infections:

Convoluted colonies.—Plated from infections 15 days to 2½ months old.

Colonies with radiating lines.—Plated from infections 8 days to 3 months old.

Concentrically striated colonies.—Isolated from infections 15 days to 2½ months old.

Plates containing no colonies with markings of any kind.—Poured from infections 8 days to 2½ months old.

All types have been obtained in the plates poured from artificial infections with *Bact. phaseoli sojense* on both soybean and Phaseolus.

GENEALOGY OF COLONIES WITH INTERNAL MARKINGS.—There is a tendency for the variously marked colonies to remain true to type after passage through the plant.

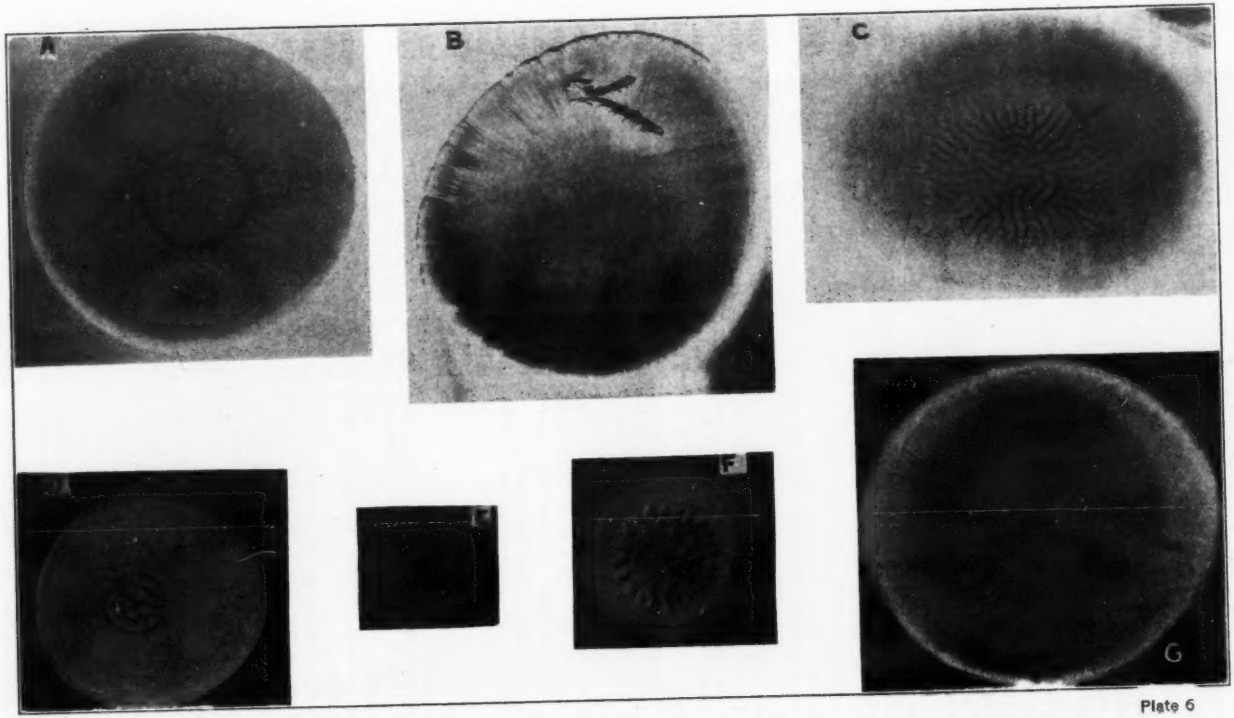
Convoluted colony 3, May 26, 1919 (Pl. 6, C).—Out of seven platings from infections produced by this colony, six contained convoluted colonies. The six platings were from four (possibly six) different plants of soybean, Lima and bush string bean. Three of these platings also contained concentrically striated colonies (Pl. 6, E), and one contained only colonies with no markings at all.

Convoluted colony 1, August 6, 1917 (like Pl. 6, A).—Five of eight platings from plants inoculated with this colony gave convoluted colonies. The five platings were from four (possibly five) different plants of soybean. Two sets of plates contained only unmarked colonies and one contained those with radiating lines (Pl. 6, B).

Concentrically striated colony 11, December 5, 1919 (like Pl. 6, E).—Two sets of plates were poured from infections produced by this colony. In both cases striated colonies were present and in one set of plates convoluted colonies also. The plates were poured from two different plants, soybean and string bean.

Colony with radiating lines.—Colony 1, April 26, 1919 (Pl. 6, B). From the single set of inoculations made with this colony only colonies of the same type and unmarked colonies have been isolated.

Colony with no markings.—Colony 6, October 20, 1919. Colonies of all the types above mentioned appeared in two sets of plates from infections with this colony. In one set of plates from soybean, convoluted, striated, and unmarked colonies were present; in the other, from wax bean, colonies with radiating lines and unmarked ones were present.



Bacterial Pustule of Soybean

Plate 6

(For explanatory legend, see p. 247)

TYPE OF COLONY IN RELATION TO VIRULENCE.—Typical infections have been obtained with all the types of colonies described. Apparently there is no relation between the colony markings and the degree of virulence.

COLONIES OF BACTERIUM PHASEOLI EFS. ON 1 PER CENT BEEF AGAR PLATES AT ROOM TEMPERATURE (PL. 7).

The type of colony most commonly appearing in isolation plates of *Bact. phaseoli* EFS. is round, smooth, wet-shining, slightly convex with thin, clear, colorless regular margins (Pl. 7, D). Frequently, however, colonies with coarse or fine concentric striæ are present (Pl. 7, A, J) and more rarely colonies with distinct radiating lines (Pl. 7, B, C); as in the case of the soybean organism, these markings are internal. Occasionally there is a lobed and fluted colony (Pl. 7, I), and there are still other types now and then (Pl. 7, K to M). In colony 7, May 9, 1919 (Pl. 7, L), the center was considerably depressed, as was that of most of the colonies on the same plate. Only twice have the colonies with internal convolutions so characteristic of *Bact. phaseoli sojense* been observed (Pl. 7, C).

As in the case of *Bact. phaseoli sojense*, the different types of colonies sometimes appeared on the same plate (Pl. 7, A, B). All colonies in Plate 7 produced infection except K and M, which were not tested. One set of comparative inoculations is illustrated in Plate 7, E to H. On August 12, 1918, Lima bean pods were sprayed with water and smeared with slime from potato cultures of August 3 of four types of colonies—striated, with radiating lines, convoluted, and unmarked (Pl. 7 ,A to D). The pods were then wrapped in oiled paper. When the paper was removed, August 19, there were water-soaked spots (Pl. 4, G), and on August 31, 19 days after inoculation, the pods were badly infected, as shown in Plate 7, E to H. In this experiment the striated and unmarked colonies were by far the most infectious, but in other inoculations the type with radiating lines has also been very virulent. The convoluted type came from a much older isolation than the others. It was isolated in Idaho in 1914, and the others in 1917. This may explain its lesser degree of virulence, since it was formerly very infectious.

MORPHOLOGY OF BACTERIUM PHASEOLI SOJENSE

Short rods with rounded ends, single or in pairs 1.4 to 2.3 μ × 0.5 to 0.9 μ. Actively motile, 1–2 polar flagella. Rods from water suspension of beef agar slants 1 to 3 days old stained with Casares-Gil's (Pl. 3, E) and van Ermengem's flagella stains. Smith describes *Bact. phaseoli* EFS. as one-flagellate (*16*).

LONGEVITY

IN CULTURES.—*Bacterium phaseoli sojense* lives on potato at room temperature three to five months provided inoculation is made on freshly made cylinders. Stock cultures in the ice box have been alive one year and four months on both potato and beef agar (stabs) in small-bore tubes (1½ cm. diameter). On the other hand, beef agar stock cultures in tubes 2 cm. in diameter did not live six months in the ice box. *Bact. phaseoli* EFS. has lived 1½ years on beef agar (small-bore tubes) in the ice box but has died in three months in the ice box when growing in beef agar in the large-bore tubes.

IN THE PLANT.—*Bact. phaseoli sojense* has been plated from soybean leaves kept dry in the laboratory for eight months, and colonies so obtained were very infectious. Very few of the organisms were alive, however, and it is often difficult or impossible to isolate the parasite from such material.

RETENTION OF VIRULENCE

Bacterium phaseoli sojense does not lose its virulence readily. Excellent infections have been obtained with a colony 1½ years after its isolation.

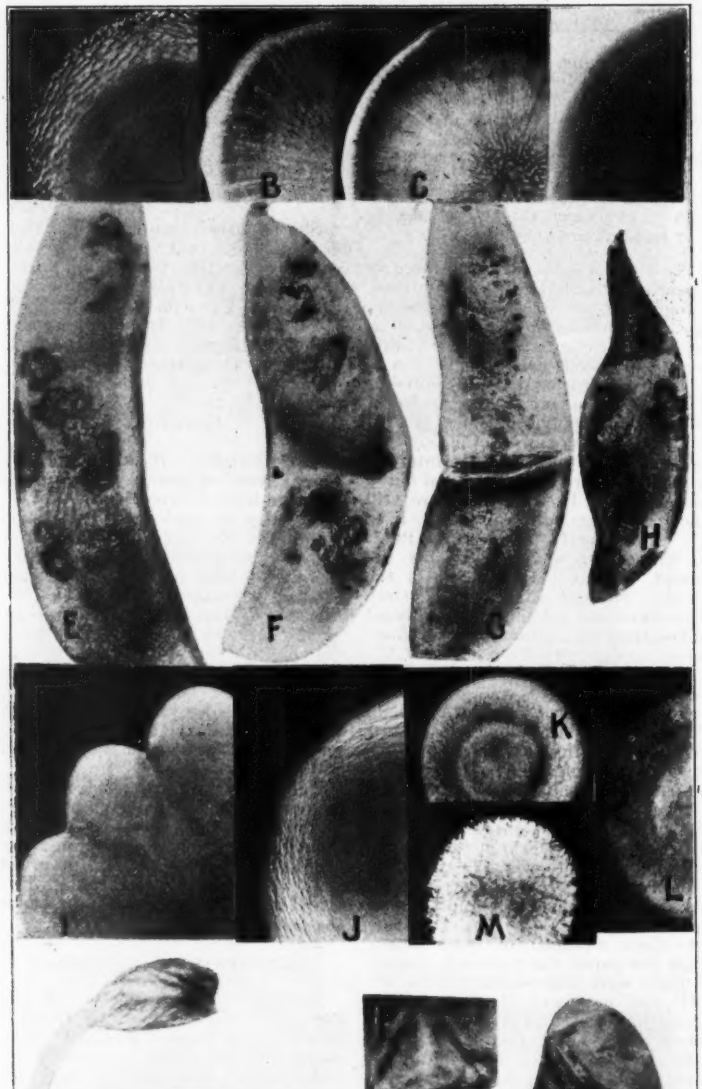
A colony of *Bact. phaseoli* EFS. produced infection on Lima bean pods 3 years 11½ months after its isolation. It was not, however, as virulent as colonies from more recent isolations.

EXPLANATORY LEGEND FOR PLATE 6

Bacterium phaseoli sojense. (Photographs by JAMES F. BREWER)

Colony types on beef agar plates, all pathogenic. ×10. All markings are internal, surface smooth.

A.—From Lima bean pod (Pl. 4, E), convoluted colony 6 days old.
B.—From pustule on soybean (Pl. 1, E), colony with radiating lines; colony 3 weeks old.
C.—From pale green spots with red centers on soybean; convoluted colony 9 days old.
D and E.—On same isolation plate, from soybean: E, concentric striæ; colonies 7 days old.
F.—From bush string bean (Pl. 4, A); colony 8 days old.
G.—From yellowish-green spot on dwarf wax bean; colony 5 days old.



Bacterial Pustule of Soybean

Plate 7

(For explanatory legend, see p. 249)

TRANSMISSION OF SOYBEAN PUSTULE

Very little is known regarding the transmission of the disease. Circumstantial evidence points to seed and perhaps insect transmission, although there is little experimental evidence.

SEED TRANSMISSION.—In the spring of 1919 some very badly diseased seed was received from Arkansas. Bacteria were found in some of the blackened spots, but all attempts at isolation failed, the plates being quickly overgrown by a spreading green fluorescent organism. Some of the seeds were then germinated and in 10 days 7 out of the 12 seedlings were badly diseased. The stems were water-soaked and full of bacteria, and 11 seedlings were broken over. *Bact. phaseoli sojense* was isolated and proved to be very infectious to Lima and bush string bean. There were many secondary infections also as new leaves unfolded on the diseased plants. No infection was obtained on Ito San soybean, however, although this is a very susceptible variety, and the plants inoculated ranged from seedlings 1½ inches high with no leaves to plants 8 or 9 inches high with leaves in all stages of development. Both spray and prick inoculations were made (inoculations of June 18 and 26, 1919).

INSECT TRANSMISSION.—Inasmuch as the disease was observed on 40 varieties of soybean growing in close proximity at Arlington Farm, Va., 1921, and raised from seed from widely separated regions,[18] it seemed reasonable to suppose that some of the seed at least was sound and that the plants became infected from their neighbors directly or indirectly. In the Dismal Swamp in 1917 the spotted cucumber beetle (*Diabrotica duodecimpunctata*) had been found on the diseased plants and a series of experiments was planned in an attempt to discover whether this insect might be a carrier of the infection. The results were negative. In the first experiments the beetles were fed on diseased leaves but they seemed to avoid the infected areas, which were rather hard and dry at this stage. Other beetles were fed on leaves smeared with *Bact. phaseoli sojense* and on potato cultures of it, which they seemed to relish, then transferred to healthy soybean plants. Although they fed on the leaves of the healthy plants, no infection resulted. It may be that temperature and moisture conditions were not favorable. The plants were in insect cages in the hothouse and no attempt was made to keep them in a moist atmosphere at any time during the experiments (carried on in October and November).

OTHER MEANS OF TRANSMISSION.—There is a possibility, of course, that the organism is spread by the rain and that it lives over on soybean refuse in the soil, but no observations have been made to support this theory.

SUMMARY

1. This paper describes a leafspot of soybean (*Glycine hispida*) produced by *Bact. phaseoli sojense* Hedges and characterized in the early stages by minute pustules and later by irregular reddish-brown spots, sometimes accompanied by yellowing.

2. *Bact. phaseoli sojense* is a short, 1-to 2-polar flagellate rod, very closely resembling *Bact. phaseoli* EFS. in most respects, but its colonies are commonly characterized by certain very striking internal markings, which are almost wholly absent from colonies of *Bact.*

[18] There were 177 different lots of seed, 124 of them having been received in 1921 and hence grown for the first time at Arlington. The seed had been obtained from Arkansas, Delaware, Georgia, Illinois, Indiana, Iowa, Kentucky, Louisiana, Maryland, Michigan, Minnesota, Mississippi, Missouri, Nebraska, North Carolina, New York, Ohio, Pennsylvania, South Dakota, Tennessee, Virginia, West Virginia, Wisconsin, China, Java (some seed received from each of the foregoing localities in 1921), Japan, and Manchuria.

EXPLANATORY LEGEND FOR PLATE 7

Bacterium phaseoli EFS. (Photographs by JAMES F. BREWER)

Colonies on beef agar plates; inoculations on Lima bean pods and soybean seedlings.

A to D.—Colonies 10 days old. (×10, except C, which is ×5.) First two are in same poured plate and descended from a colony like type A.

E to H.—Lima bean pods six weeks after inoculation with colonies A to D, respectively. ×1.

I to M.—Rarer types, except J, which is frequent. (J is 6 days old.) L has depressed center. All except K and M, tested and found pathogenic. ×5–10.

N and O.—Germinating soybean seed sprayed in damp chamber with *Bact. phaseoli* EFS. Sown in damp chamber November 24, 1920, inoculated November 26, 1920, and photographed eight days later; water soaking and bacterial ooze on cotyledons; organism reisolated. ×3 ca.

P—Browned and curled leaf tip from soybean seedling No. 27 grown from seed germinated in damp chamber (sown November 24, 1920), transferred to Sachs' solution December 1, sprayed in greenhouse December 2, with *Bact. phaseoli* EFS.; organism reisolated from similar leaves. Photographed eight days after inoculation. ×2.

phaseoli EFS. Furthermore, soybeans are not readily infected by *Bact. phaseoli* EFS.

3. *Bact. phaseoli sojense* does not produce pustules on Phaseolus. On the other hand, the best infections on the latter are not to be distinguished from those caused by *Bact. phaseoli* EFS.

4. The pustules are caused by both hypertrophy and hyperplasia chiefly of the parenchyma.

5. *Bact. phaseoli sojense* is easily isolated from fresh material by the poured-plate method. It has also been isolated from herbarium material 8 months old.

6. Artificial infection has been obtained by pure culture inoculation on a number of varieties of soybean, and natural infection has been observed on 40 varieties in experimental plats at Arlington Farm, Va.

7. Successful inoculations have been made on Lima, string, and wax beans of the genus Phaseolus.

8. There is some evidence that passage of *Bact. phaseoli sojense* through Phaseolus increases its virulence for the same.

9. In comparative inoculations on Phaseolus with *Bact. phaseoli sojense* (directly from soybean) and *Bact. phaseoli* EFS. the latter was much more infectious.

10. *Bact. phaseoli* EFS. is only very weakly pathogenic to soybean plants. Only once did infection occur in a long series of hothouse inoculation experiments made according to the method which was so successful with this organism on Phaseolus, or with *Bact. phaseoli sojense* on Phaseolus or soybean. In this exceptional case infection was very slight and did not resemble that caused by *Bact. phaseoli sojense* on soybean. Brown spots and streaks but *no pustules were produced*. The organism was reisolated.

11. *Bact. phaseoli* EFS. produced infection on soybean seedlings (1) grown from seed treated with formaldehyde or sulphuric acid and germinated in damp chamber and inoculated therein immediately after germination; (2) grown from formaldehyde-treated seed, germinated in damp chamber, transferred to Sachs' solution as soon as the seedlings had made sufficient growth, and inoculated eight days after sowing; (3) growing in pots from formaldehyde-treated seed germinated in damp chamber and sprayed in inoculating cages when they had developed the first pair of leaves, the leaves being rubbed between the thumb and forefinger after the spraying. No infection on leaves not so rubbed. (4) Grown from seed treated with sulphuric acid, washed, dipped in a water suspension of *Bact. phaseoli sojense* and germinated in damp chamber. By far the best infections were obtained in (1). *In none of these experiments were pustules formed.*

12. With the exception of the colonies on beef agar plates *Bact. phaseoli sojense* behaves on all the culture media tested like *Bact. phaseoli* EFS. Some additional cultural work was done with the two organisms. Some of the most favorable media are steamed potato cylinders, whey agar, potato agar plus 10 per cent dextrose, and Kellerman's synthetic agar plus Congo red.

13. Neither *Bact. phaseoli sojense* nor *Bact. phaseoli* EFS. loses its virulence readily, and both can be kept alive in beef agar stabs in the ice box for a year or more if small-bore (1½-cm.) tubes are used.

14. The disease is known to occur in Texas, Virginia, Louisiana, South Carolina, Kansas, Delaware, and Arkansas.

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VITAMIN A CONTENT OF FRESH EGGS[1]

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The significance of vitamins from the general standpoint of health and nutrition is now so well established that all new data on their content in foods are of prime importance. Several characteristic diseases and functional disorders are associated with a lack or deficiency of specific vitamins in the diet. Among these are beriberi, xerophthalmia, scurvy, rickets, impairment of the process of reproduction, failure of growth in young animals, and decline in weight in adults. The different vitamins are so distributed in our common food products that the diet of the average person probably contains an adequate supply. Nevertheless, through force of circumstances or on account of individual preference, many persons live on a diet so restricted that these food accessories are not obtained in sufficient quantities to meet nutritional requirements or to insure the optimum of health and well-being. This is particularly true in the case of small children. Cramer (*3*)[2] has recently pointed out that a borderland between health and disease may be created by a restriction in the vitamin supply. A diet may contain enough vitamins to afford protection from obvious ill health or interference with the breeding and rearing of the stock, and yet be so restricted as to lead to imperfect development and deviations from the optimum which up to the present have been overlooked.

It is becoming increasingly recognized that deficiency diseases and disorders caused by an insufficient intake of vitamins are of more frequent occurrence than has been generally supposed. Cramer (*3*) refers to outbreaks of an eye affection in England which have been ascribed to a lack of sufficient vitamin A and which were eliminated by the administration of cod liver oil. An account has also recently been given by Bloch (*1*) of a surprisingly large number of cases of xerophthalmia among children in Denmark who had not been getting sufficient vitamin A. The large number of cases of malnutrition among children in our public schools, and the beneficial effects produced by the recently introduced practice in many schools of furnishing milk, emphasize the need of greater attention to the qualitative and quantitative composition of the dietary.

It has been aptly stated that "vitamins should be obtained from the dairy, the grocery and the market, not from the drug store." Among the best known sources of vitamin A are cod-liver oil, butterfat, spinach, tomatoes, and egg yolk. From the standpoint of the health and nutrition of the general public the importance of any particular foodstuff as a source of vitamin must be considered from certain other angles as well as from its vitamin content. Of two substances having the same vitamin content, that one will be of the greater general importance which is the less expensive; which is more readily available to all people at all seasons and in all places; which combines with its vitamin value the greater food value with respect to other dietary factors; which is the more digestible, the more palatable, and better tolerated by infants and invalids. In accordance with these considerations, eggs must be considered a very important, if not the most important, source of vitamin A. Eggs and milk are the two articles which in themselves come nearest to being perfect foods. They contain all of the dietary factors essential for the nutrition of an animal during the early stages of its existence.

Hess (*4*) found that egg yolk is well tolerated by babies. He reported that for six months about 50 babies were fed with excellent results a mixture consisting of milk (24 oz.), barley water (12 oz.), sugar (¾ oz.), and 1 egg yolk; and he states that "egg yolk possesses marked antirachitic properties—far more than any other natural food product (*5*)." That egg yolk is a rich source of vitamin A was pointed out by Osborne and Mendel (*7*) and by McCollum and Davis (*6*) in their early work on this vitamin. Working with the yolk of fresh eggs, Hess found that 0.25 gm. fed daily was sufficient to protect rats from rickets. Casparis,

[1] Received for publication July 11, 1924; issued January, 1925. This paper was presented at the 67th meeting of the American Chemical Society held in Washington, D. C., Apr. 21 to 26, 1924.

[2] Reference is made by number (italic) to "Literature cited," p. 257.

Shipley, and Cramer (*2*) obtained similar results. Hess states that a smaller quantity than 0.25 gm. of egg yolk would probably have been sufficient; but no attempt was made to force the feeding to a minimum.

EXPERIMENTAL

COMPOSITION AND PREPARATION OF DIET

The experiments described in this paper were undertaken to ascertain, as nearly as present methods permit, the smallest quantity of fresh whole egg which will cure rats of xerophthalmia, and enable them to grow at a normal rate.

The eggs used were laid during the summer months, and were from hens that had access to plenty of fresh, green food.

Young albino rats weighing from 45 to 55 gm. were placed on a basal ration found experimentally to be practically free from vitamin A, which had the following composition: Casein, 15 per cent; corn starch, 65 per cent; Crisco, 15 per cent; salt mixture, 5 per cent. Rats on this diet usually developed xerophthalmia in 40 to 50 days.

Vitamin B was supplied by 0.2 gm. of a commercial autolysed yeast preparation (marmite) fed separately each day.

The casein was prepared by precipitation at its isoelectric point from fresh skimmed milk, and purified by dissolving it in dilute sodium hydroxide and reprecipitating with acetic acid. This treatment was followed by extraction with alcohol and ether. The casein was then dried at 110° C. The starch was not specially treated, since it was not found to contain a detectable quantity of vitamin. Air was bubbled through the Crisco at about 100° for 48 hours, to insure the destruction of any vitamin which may have been present. The salt mixture used was that described by Osborne and Mendel (*8*). The egg was thoroughly beaten to a homogeneous consistency, and fed daily together with the marmite, separate from the rest of the diet.

POTENCIES OF VARIOUS QUANTITIES OF THE WHOLE FRESH EGG FOR THE PROMOTION OF GROWTH AND CURING OF XERPHTHALMIA

No curative dosage of egg was given until the rats had developed definite xerophthalmia. On a vitamin A free basal diet young rats usually make an initial growth at a fair rate for a few weeks and then cease to grow or begin to decline in weight. Many investigators begin to feed the material to be tested at this point, and note the effect on the growth of the animals as an indication of the vitamin potency of the material. Sometimes the first decline in weight is but temporary, and growth for a short period again follows, even without the addition of any vitamins. But waiting until xerophthalmia is evident before giving the substance to be tested may not only prevent a possible error due to this phenomenon, but permits a study of the effect of the vitamin A both as an antixerophthalmic and as a growth-promoting factor. These functions may be distinct and not strictly parallel.

The first trials were made with 0.5 gm. of egg given daily after the onset of xerophthalmia. The results are shown in Figure 1. As can be seen, the feeding of the egg promptly arrested the decline in weight, and enabled the rats to grow at a very fair, although not normal, rate. Xerophthalmia, which was very severe in the case of rats No. 1783 and No. 1785, was entirely cured in from 10 to 25 days and at the close of the experiment all the rats were in very good condition.

After it was found that 0.5 gm. of egg daily would cure xerophthalmia, without, however, permitting normal subsequent growth, several rats were fed 0.75 gm. of egg (fig. 2). The response to this was prompt. Rat No. 1794 developed an abnormality of tooth growth which interfered with feeding, and after an initial period of rapid gain had to be removed from the diet. Rat No. 1857, after having been given the egg, grew at a rate far exceeding normal. The eyes of all cleared up within a week.

It is evident that 0.75 gm. daily of fresh egg was sufficient to bring about normal growth in rats which had declined in weight and had developed xerophthalmia as a result of a lack of vitamin A in the diet. Growth at a rate of only about two-thirds of normal was secured on 0.5 gm. of egg. The minimum quantity of egg required to admit of normal growth can not, however, be safely decided from these experiments, since the rats were suffering badly from avitaminosis when the egg feeding was started. Consequently, more egg might have been necessary to permit subsequent growth at a normal rate than would have been the case had they received the egg from the beginning of the experiment. In another series of experiments in which 0.5 gm. of egg was fed daily from the beginning to young rats weighing about 50 gm., growth at the normal rate was secured for about 100 days, after which the growth became subnormal. From

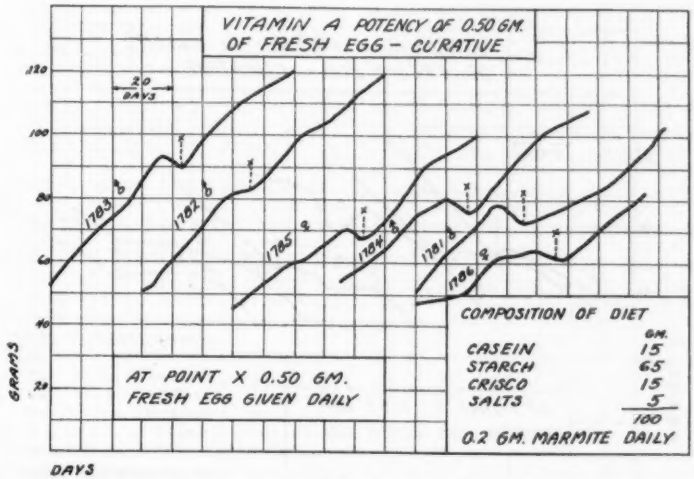

FIG. 1.—Vitamin A potency of 0.50 gm. of whole fresh egg. Beginning at the points marked "x," the curative dose of egg was given daily after the rats had shown unmistakable symptoms of xerophthalmia

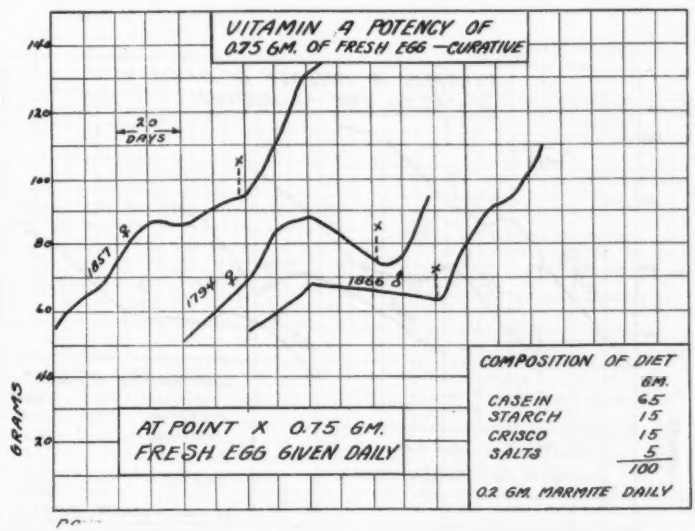

FIG. 2.—Vitamin A potency of 0.75 gm. of whole fresh egg. Beginning at the points marked "x," the curative dose of egg was given daily after the rats had shown unmistakable symptoms of xerophthalmia

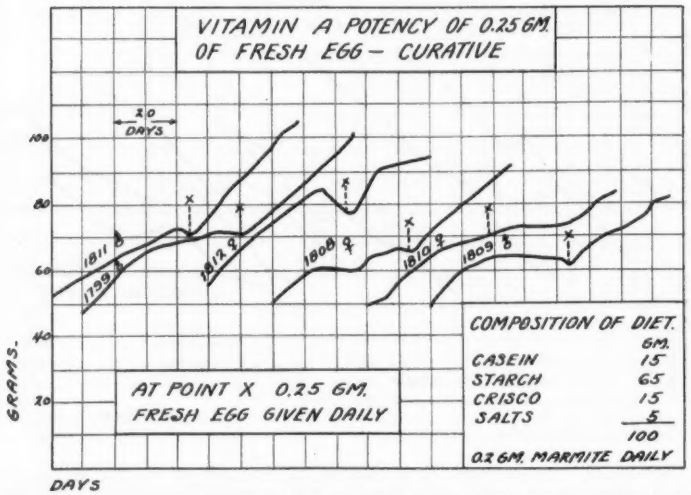

FIG. 3.—Vitamin A potency of 0.25 gm. of whole fresh egg. Beginning at the points marked "x," the curative dose of egg was given daily after the rats had shown unmistakable symptoms of xerophthalmia

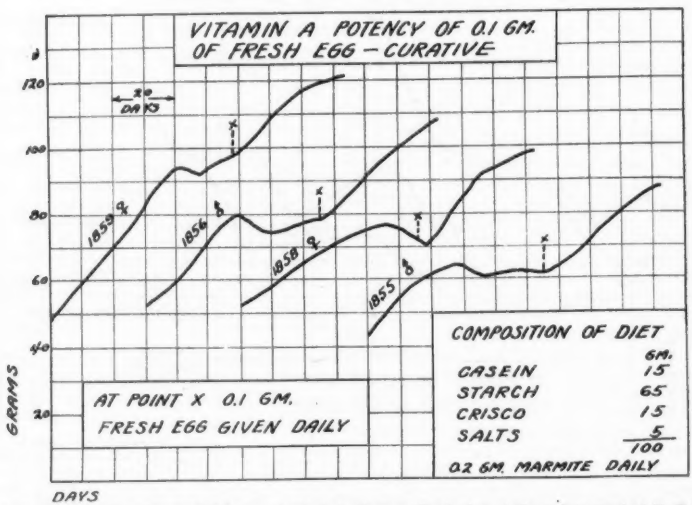

FIG. 4.—Vitamin A potency of 0.1 gm. of whole fresh egg. Beginning at the points marked "x," the curative dose of egg was given daily after the rats had shown unmistakable symptoms of xerophthalmia

these results it would appear that the minimum quantity of vitamin A required for the normal growth of rats is furnished by an amount of fresh whole egg very little more than 0.5 gm.

Since 0.5 gm. of egg was found ample to cure xerophthalmia, smaller amounts were tried, to determine the minimum dosage that could be used. Accordingly, six rats were fed 0.25 gm. of egg. The results in this case were decidedly poorer than those with the 0.5 gm. dose (fig. 3). Growth was slightly less, and the length of time required to cure the sore eyes was very much increased. One rat, No. 1808 while showing fair growth, still exhibited distinct signs of sore eyes at the close of the experiment, 38 days after the beginning of the egg feeding.

The lower limit seemed to have been reached at 0.25 gm., but another series was tested with 0.1 gm. of egg (fig. 4). Although the growth recovery of these was not far different from that of those receiving 0.25 gm., the effectiveness of 0.1 gm. for curing xerophthalmia was found to be very slight. Only one rat, No. 1856, was completely cured after three weeks of feeding; and the others, even after six weeks, gave no indication that they would ever recover fully on this dosage.

The fact that the weight increase of the rats receiving 0.1 gm. of egg was nearly identical with that of those receiving 0.25 gm., and not far below that of those receiving even 0.5 gm., is a somewhat surprising phenomenon which is being studied by the authors.

DISCUSSION

These experiments indicate that test rats, after having declined in weight and having developed xerophthalmia as a result of vitamin A deficiency, can be restored to normal weight by feeding 0.5 to 0.75 gm. of whole egg daily; and that 0.5 gm. is very near the quantity required to enable healthy young rats to maintain growth at a normal rate. If weight increase be not considered, but only the effectiveness for curing xerophthalmia, the lower limit is reached at about 0.25 gm. Only one rat failed of complete cure on this dosage, and this one might possibly have recovered eventually.

It is of interest to calculate these figures in terms which are sometimes used by other workers. Since the yolks compose about 35 per cent of the whole egg, 0.75 gm., 0.50 gm. and 0.25 gm. of whole egg would then be equivalent to 0.26 gm., 0.17 gm., and 0.088 gm. respectively, of yolk. The minimum quantity (0.25 gm. of yolk) which Hess found sufficient to protect rats from rickets was, therefore, very close to the minimal growth-restoring dose. Of the whole yolk 49.8 per cent is water. The quantity of egg yolk required, calculated to a dry basis, would therefore be between 0.083 gm. and 0.13 gm. for growth restoration, and 0.041 gm. for the cure of xerophthalmia. On the assumption that all of the vitamin A is contained in the oil of the yolks, then, since 60 per cent of the dried yolk is oil, a little more than 0.05 gm. of egg oil would be required for growth promotion, and 0.025 gm. would be required for the curing of xerophthalmia. Cod-liver oil has been found effective in doses of about 0.001 gm. Therefore egg oil would have a vitamin A potency between 2 and 4 per cent of that of the most potent cod-liver oil.

SUMMARY

Feeding experiments have shown that from 0.50 gm. to 0.75 gm. of fresh, whole egg fed daily supplies young rats with sufficient vitamin A for growth at a normal rate. A smaller quantity, 0.25 gm, was found to be adequate to cure well advanced cases of xerophthalmia.

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SOME INSECTICIDAL PROPERTIES OF THE FATTY ACID SERIES [1]

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The use of soaps as contact insecticides is of long standing in economic entomological practice, although there has apparently been no serious investigation leading toward the determination of the active principle chemically responsible for their value as such. References available to the writers attribute the toxicity of soaps to their alkaline ingredients, expressing the belief that their other constituents, the fatty acids, are practically inert in their action toward insects. In the belief that the results obtained through an investigation of this problem during the past year justify their presentation for the consideration of other investigators, this brief preliminary paper, including some of the theoretical aspects and a promising practical application to insecticide entomology, is herewith presented.

Soaps have been defined as the alkali salts of the fatty acids. Since those most commonly in use are formed from the fatty acids containing an even number of carbon atoms united in a straight chain, or the normal, saturated monocarboxylic fatty acids found in nature, this series was chosen as the subject of the tests first conducted, as herein outlined. A complete series of the even-carbon acids through stearic acid was tested, several of the odd-carbon acids being likewise compared. When it was possible to obtain them, purified acids were used, although the technical grades were employed when necessary. The following homologues of the series $C_NH_{2N}O_2$ were available: Acetic, propionic, butyric, valeric, caproic, oenanthylic, caprylic, pelargonic, capric, lauric, myristic, palmitic, and stearic acids.

The results obtained on the available species of aphids were striking. While no great toxicity has been encountered with the lower homologues, the caproic acid showed marked toxicity, and tests of the next in the series, caprylic acid, were gratifying. Kills of more than 90 per cent of the black chrysanthemum aphid were attained at 1 to 500 dilutions, with only slightly decreased results when the water proportion was increased to 1 to 1,000. Capric acid, the next even-chain homologue, killed more than 99 per cent of the green apple aphid at a dilution of 1 part to 1,200 of water. Lauric acid at the same dilution was fatal to 92 per cent of the same aphid, while myristic acid at 1 to 1,200 killed 78 per cent of the same species.

Paralysis is complete and practically immediate when aphids are subjected to toxic strengths of the fatty acids. It was noted in tests on the black chrysanthemum aphid in comparison with nicotine sulphate (commercial 40 per cent) that in the case of the latter poison the mortality was indicated by the large percentage of dead aphids which dropped from the sprayed plants. In the case of the fatty acids, the dead insects remained attached by their inserted beaks, affording no gauge of toxicity, such as was shown by the nicotine, until the actual counts were made.

The fatty acids tested were applied in the free form, and as acid, neutral, and alkaline soaps, using potassium, sodium, and ammonium bases. In the free form, they were emulsified by means of various stabilizers, and when used with distilled water alone, by means of a colloid mill. In the later part of the investigation a solution of glue was used as a standard emulsifier, providing ample stability for uniform experimental tests. Throughout the study parallel tests, using a commercial 40 per cent nicotine sulphate solution in combination with soap, were conducted for comparison, and ample check or untreated material was kept under observation.

Practically all tests were made on a laboratory scale in an outdoor insectary. The insects were thoroughly wetted by means of a small atomizer drawing from the bottom of the chamber.

It is hoped that at a somewhat later date detailed information as to the results of the season's work may be presented.

[1] Received for publication Sept. 12, 1924—issued January, 1925.

The principal subjects of the tests have been the rosy apple aphid, *Anuraphis roseus* Baker, the green apple aphid, *Aphis pomi* DeGeer, the bean aphid, *Aphis rumicis* Linn., the black chrysanthemum aphid, *Anuraphis sanborni* Gill., the black cherry aphid, *Myzus cerasi* Fab., and the aster aphid, *Macrosiphum ambrosiae* Thos. Preliminary tests further indicate that this material is toxic to insects of other orders as well as to Acarina.

The physical characteristics of the fatty acids tested lend themselves admirably to use as contact insecticides. The fatty acids showed unusual wetting and spreading powers when applied in the emulsion form to either insects or foliage, wetting readily such insects as the polished black aphids, the squash bug, and hairy caterpillars. Leaves having a waxy covering, such as nasturtium and cabbage, are easily and evenly coated. The physiological action of the fatty acids on the insect organism is as yet undetermined.

Experiments so far conducted indicate that the toxicity of the fatty acids increases with the molecular weight, at least to a certain point not yet definitely determined. A practical amount of toxicity was reached with the sixth carbon atom, the peak of toxicity apparently lying at or slightly above the C_{10} point. Toxicity is much greater in acid mixtures, the free acids killing at materially higher dilutions than when combined as their corresponding soluble alkaline or neutral salts. In contradistinction to the action of nicotine, no variation in toxicity is experienced under varying summer temperatures and humidity, leading to the conclusion that the low volatility of the fatty acids insures their retention in the body of the insect until action is complete.

Plants show a varying susceptibility to burning by the acids of this chemical series. The foliage of the apple is not affected when sprayed with strengths toxic to apple aphids. No injury to chrysanthemum plants was shown by dilutions fatal to the black chrysanthemum aphid. On the other hand, the foliage of nasturtium is injured by the dilutions required to kill the bean aphid.

Tests toward the practical application of the insecticidal properties shown by preliminary work with the long-chain fatty acids resulted in the selection of a product commercially known as "double distilled coconut fatty acids" as embodying in the greatest degree the desirable features of those members of the series approximating the peak of practical toxicity. A typical sample of this product, much used industrially in soap manufacture and otherwise, was fractionated [2] with the following results:

	Per cent.
A.—Fraction mostly caprylic acid (small amounts caproic and capric)	4. 0
B.—Fraction mostly capric acid (small amounts caprylic and lauric)	14. 6
C.—Fraction mostly lauric acid (small amounts capric and myristic)	51. 1
D.—Fraction mostly myristic acid (small amounts lauric and palmitic)	14. 1
E.—Fraction mostly palmitic acid (small amounts myristic, oleic, and stearic)	8. 3
F.—Fraction mostly oleic and stearic acids (small amount palmitic)	5. 6
	97. 7

The fractions A to D as shown were individually tested for contact toxicity, fraction B, containing mostly capric acid, showing slightly greater toxicity than either A, C, or D. This tends to confirm the theory that the peak of toxicity lies near capric acid $C_9H_{19}COOH$.

The commercial product selected is liquid at usual summer temperatures (M. P. 27° C.), highly toxic even when greatly diluted, stable with lubricating-oil emulsions, readily obtainable in quantity, and at the time of writing is much lower in price than nicotine. Difficulties in the preparation of a stock solution and the retention of the insoluble acids in a stable emulsion have been met by the addition of an equal amount of benzol gasoline to the commercial fatty acids, afterwards using powdered glue as a colloidal stabilizer. The gasoline lowers the melting point to 5° C., thus serving to prevent solidification of the acid globules in the emulsion through lowered temperatures, also facilitating even distribution, while the glue greatly retards separation after emulsification. The stock emulsion [3] has been tentatively

[2] This work was done by the Insecticide Laboratory of the Bureau of Chemistry, United States Department of Agriculture.

[3] *Stock solution*

Coconut fatty acids (double distilled)	200 cc.
Gasoline (benzol)	200 cc.
Glue (granular)	100 gm.
Water	525 cc.

adopted as a practicable basis for further tests. In series of tests with this formula, used in the proportions of 1 part of the coconut fatty acids to from 800 to 1,200 parts of water, a mortality rate has been attained against the green apple aphid of from 94 to 98 per cent, equivalent to the rates obtained with commercial 40 per cent nicotine sulphate in parallel tests at the same dilutions.

Approximately a liter of stock solution is obtained when made up as indicated, each 5 cc. containing 1 cc. of the commercial mixture of acids. Upon thorough shaking it readily becomes a milky emulsion, capable of dilution with any ordinary proportion of water, and when so diluted it remains sufficiently stable for practical application. The spreading power of the emulsion is excellent, toxicity high, and cost of material extremely low. Prepared by this formula, coconut fatty acids have compared favorably in efficiency, pound for pound, in the experiments so far conducted, with commercial nicotine preparations at less than one-fourth the cost per gallon of spray mixture.

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